THE EFFECTS OF NUTRIENT AVAILABILITY ON THE HOST PLANT RESISTANCE OF GERBERA TO WESTERN FLOWER THRIPS

A Dissertation

by

JAMES DAVIS SPIERS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2007

Major Subject: Horticulture

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Approved by:

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ABSTRACT

The Effects of Nutrient Availability on the Host Plant Resistance of Gerbera to Western Flower Thrips. (December 2007)

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Nutrition of host plants has been shown to have a direct effect on the productivity of numerous insect pests, including western flower thrips [(WFT) *Frankliniella occidentalis* (Pergande)] – a major pest on both horticulture and agronomic crops. Plants use constitutive and induced chemical defenses to aid in protection against phytophagous insects. Reductions in WFT abundance in response to decreased nutrient availability has been attributed to the reduced availability of nutrients required for WFT productivity. The goals of this research were to determine the effects of fertilization on chemical defenses, and subsequent effects on WFT feeding and abundance. More importantly, the effects of fertilization and WFT feeding on plant growth, development, physiology, and quality were determined to assess the viability of optimizing fertilization in order to increase host plant resistance in gerbera.

Constitutive (i.e. phenolics) and induced (i.e. jasmonic acid) chemical defenses were enhanced when fertilization was reduced. Reducing fertilization increased the total phenolics and wound- and WFT-induced jasmonic acid (JA) accumulation in gerbera. The enhanced chemical defenses in lower fertility plants resulted in reduced WFT

abundance and feeding damage. These results indicate that the strategy for some plant species under nutrient stress is to increase constitutive defenses, while maintaining, or possibly increasing inducible defenses instead of growth. Similar to 0X fertility plants (only supplied with initial fertilizer charge in commercial media), 0.3X (received 30% of recommended rate) gerberas had reduced biomass and greater chemical defenses compared to 1X plants, but these plants did not appear to be nutritionally stressed—and 0.3X plants without WFT were rated as marketable. Reducing fertilization by 70% (0.3X) did not affect flower dry mass (DM) or the rate of flowering, but the flower stalks (peduncles) were taller in response to the fertilizer reduction. Hence, reducing fertilization to a moderate level in gerbera production may reduce susceptibility to WFT, while producing marketable crops.

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CHAPTER I

INTRODUCTION

A basic tenet of greenhouse crop production is to create ideal growing conditions to rapidly produce plants that are aesthetically pleasing. Water and fertilization are rarely limited inputs, and often fertilizer recommendations are high in order to prevent nutrient deficiencies and reduce production time. As part of best management practices (BMP), growers are encouraged to reduce fertilizer usage in order to reduce fertilizer run-off. Often there is near zero tolerance for insect pests on ornamental crops, therefore multiple applications of insecticides are generally required to prevent insect infestations. Reducing these pesticide applications could reduce health risks, chemical phytotoxicity, the occurrence of insecticide resistance, and production costs.

In addition to environmental concerns, high fertility regimes may compromise a plant's natural defense mechanisms and increase susceptibility to insect herbivores. Many insect herbivores are more prolific on plants treated with higher nitrogen fertility (Inbar et al., 2001). The growth and reproduction of phytophagous insects is often limited by the nutritional content of their hosts, and generally increases as foliar nitrogen content increases (Mattson, 1980). High fertility has been shown to increase the productivity of western flower thrips [(WFT) *Frankliniella occidentalis* (Pergande)]; a

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major pest on both horticulture and agronomic crops (Brodbeck et al., 2001; Davies et al., 2005; Schuch et al., 1998; Stavisky et al., 2002). The population increase was generally attributed to the higher nutritional quality of these plants for thrips utilization. Few studies, if any, have looked at the effect of fertilization on host plant resistance to WFT.

Plants use constitutive and induced defenses to protect themselves from herbivore attack. Constitutive defenses include stored allelochemicals that can reduce the attractiveness of plants to herbivores. Many of these metabolites can decrease tissue digestibility and/or toxicity to herbivores and influence herbivore feeding, oviposition, growth and development, fecundity, and/or fertility (Walling, 2000). These natural secondary metabolites are major determinants of plant resistance to herbivores (Berenbaum, 1995). Physiological constraints imposed by resource availability clearly influence allocation to secondary metabolism in plants. The quantity and quality of nitrogen available to the plant has been shown to influence the constitutive levels of a wide variety of types of secondary metabolites, including glucosinolates (Hugentobler and Renwick, 1995), cardenolides (Hugentobler and Renwick, 1995), phenolics (Inbar et al., 2001; Stout et al., 1998; Wilkens et al., 1996), alkaloids (Baldwin et al., 1993), and furanocoumarins (Zangerl and Berenbaum, 1995). Tomato plants treated with low nitrogen had twice the total phenolic content as compared to high nitrogen plants, with the highest levels of phenolics in young leaves (Stout et al., 1998). In a separate study, increases in defensive compounds (peroxidase and total phenolics) due to nitrogen

deficiency was shown to negatively affect feeding and oviposition of various insect herbivores in tomato (Inbar et al., 2001).

High rates of fertilization decrease the foliar concentration of phenolics and other secondary metabolites that provide trees with insect resistance (Bryant et al., 1983; Mattson, 1980). The growth/differentiation balance hypothesis (GDBH) attributes this response to a physiological trade-off between primary and secondary metabolic pathways (Herms and Mattson, 1992). When fertilization stimulates growth, it may divert sources from other processes, including secondary metabolism. Plants have limited resources to support their physiological processes and not all requirements can be met simultaneously. Hence, trade-offs occur among growth, storage, reproduction, and defense (Bazzaz et al., 1987; Chapin, 1991; Herms, 2002; Lambers and Poorter, 1992). While it is evident that fertilization influences host plant resistance in many systems, it is still unclear how host plant resistance and specific secondary metabolites are affected in ornamental greenhouse crops.

The phytohormone jasmonic acid (JA) is known to regulate many plant responses, including inducible defenses against insect herbivory. Herbivores that cause extensive tissue damage induce changes in plant gene expression and accumulation of secondary metabolites similar to mechanical wounding (for reviews, see Gatehouse, 2002; Kessler and Baldwin, 2002; Ryan, 2000; Walling 2000). Many proteins and secondary metabolites that accumulate after wounding and JA-treatments interfere with insect feeding, oviposition, growth and development, and fecundity (Duffey and Stout,

1996; Li et al., 2002) or attract herbivore predators (Dicke et al., 1999; Páre and Tumlinson 1999).

Jasmonates are synthesized from linolenic acid via the octadecanoid pathway (Schaller, 2001; Vick and Zimmerman, 1984). The octadecanoid signaling pathway has been shown to be essential for plant defense against chewing insects (Howe et al., 1996; Lightner et al., 1993), and more recently cell content-feeding herbivores (Li et al., 2002). Li et al. (2002) used transgenic plants that constitutively activated the octadecanoid pathway to confer resistance to attack by spider mites (*Tetranychus urticae* Koch) and WFT. In other studies, exogenous JA applications negatively affected WFT abundance and feeding (Omer et al., 2001; Thaler et al., 2001).

Recently, nitrogen deficiency was shown to increase volicitin-induced volatile emission and jasmonic acid accumulation in maize (Schmelz et al., 2003b). Volicitin, N-(17-hydroxylinolenoyl)-L-glutamine, is a nonenzymatic elicitor of plant volatile emission identified from beet armyworm (*Spodoptera exigua*) oral secretions (Alborn et al., 1997). Nitrogen-deficiency resulted in elevated and prolonged increases in wound- and volicitin-induced JA levels in maize (Schmelz et al., 2003b). However, the opposite was found in the native tobacco*, Nicotiana attenuata*. Both constitutive and induced JA levels were reduced in low nitrogen *N. attenuata* plants compared to high nitrogen plants (Lou and Baldwin, 2004). Also contrary to maize, nitrogen supply does not influence the elicited release of volatiles in *N. attenuata*. To my knowledge, these are the only two species that the effect of nitrogen supply on JA accumulation has been determined. It would be of interest to determine the trend with a commercial, floriculture crop.

Gerbera jamesonii is an economically important ornamental crop that is sold as a bedding plant and/or cut flower. Gerberas are highly susceptible to WFT and unlike agronomic or other field crops, there is near zero tolerance for insect pests in greenhouse crop production of gerbera. The fact that it is also used as a cut flower allows for more lenient insect pest control (greater opportunity to reduce N to an acceptable level for plant growth and still provide an increase in resistance to herbivorous insects). Host plant resistance mechanisms of gerbera have been investigated in response to JA and feeding damage by spider mites (*Tetranychus urticae*). Treatment of gerbera leaves with JA or feeding damage by spider mites induces the production of a complex odor blend that attracts the predatory mite *Phytoseiulus persimilis* (indirect plant defense) (Gols et al., 1999; Gols et al., 2003).

This research was conducted to determine if fertilization has a significant effect on host plant resistance characteristics of gerbera, and whether altering fertilization could be a method employed for use in an integrated pest management system in gerbera production. The general objectives and hypotheses for this research were as follows:

1. To study the influence of fertilization on the constitutive and wound-induced levels of total phenolics and jasmonic acid in *Gerbera jamesonii*.

   Hypothesis: Increasing fertilization will negatively affect both constitutive and wound-induced levels of total phenolics and jasmonic acid present in the leaf tissue of *Gerbera jamesonii*.

2. To evaluate the effect of fertilization on western flower thrips [*Frankliniella occidentalis* (Pergande)] feeding on the leaf tissue of *Gerbera jamesonii* and the subsequent effect on the accumulation of endogenous jasmonic acid.

   Hypothesis: Western flower thrips will exert more feeding damage on leaf tissue of *Gerbera jamesonii* grown under higher fertilization regimes, and western flower thrips feeding damage will induce the endogenous accumulation of jasmonic acid.

3. To evaluate the effects of fertilization on western flower thrips [*Frankliniella occidentalis* (Pergande)] abundance on potted *Gerbera jamesonii*; and the subsequent effects of fertilization and western flower thrips feeding on plant growth and development, gas exchange, leaf chlorophyll content, total phenolics, jasmonic acid, and overall quality of potted *Gerbera jamesonii*.

   Hypotheses: Western flower thrips abundance will increase as fertilization is increased. Jasmonic acid and total phenolics content will increase in plants inoculated with western flower thrips. Constitutive levels of total phenolics and jasmonic acid will be higher in low fertility plants. Fertilization will enhance plant growth and development, gas exchange, leaf chlorophyll content; and western flower thrips abundance will have a detrimental effect on those same parameters.

CHAPTER II

LITERATURE REVIEW

Host Plant Resistance to Insect Herbivores

Plants and insect herbivores have been competing for millions of years. The plant-herbivore interaction has influenced species diversity in both host and herbivore (Harborne, 1988). Plants and herbivores have evolved and developed ways to co-exist. Some plants attempt to reduce herbivore damage through rapid growth and development, or adapting to other habitats. Other plants produce and accumulate defense compounds to deter herbivory. In spite of the diversity of the plant kingdom, plants in general possess a defensive mechanism in response to wounding that can be effective against many insect herbivores.

Plants may use different strategies to protect against insect feeding. Direct defenses are any plant traits (e.g., thorns, silica, trichomes, primary and secondary metabolites) that by themselves affect the susceptibility to and/or the performance of attacking arthropods and thus increase plant fitness in environments with herbivores. Indirect defenses are plant traits that attract predators and parasitoids of herbivores and increase the carnivore's foraging success and thereby facilitate top-down control of herbivore populations (Kessler and Baldwin, 2002). If these defenses occur naturally without requiring a stimulus, then they are said to be "constitutive" plant defenses.

Conversely, if the defense requires activation, then they are described as “induced” plant defenses.

Constitutive Plant Defenses

Host plant resistance results from traits that reduce herbivore growth, fecundity, and survival (antibiosis), the attractiveness of plants to herbivores (antixenosis), and/or increase the ability of plants to compensate for herbivory (tolerance) (Painter, 1951; 1958). In some cases, herbivore tolerance may be compensated for by increased plant growth in response to herbivore pressures (Herms and Mattson, 1992). Secondary metabolism, which involves specialized, often complex and species-specific biosynthetic pathways, is thought to provide compounds which are accumulated and stored, so that when attacked, the plant is already provided with the means to deter or kill herbivores. Plants may possess constitutive defenses that can act as a physical barrier, as in lignification or resin production, or can act as a biochemical signal perceived by the herbivore, as in deterrents of feeding or egg deposition, or can act as a toxin. Toxic compounds (e.g., alkaloids, terpenoids, phenolics) poison generalist herbivores, forcing specialists to invest resources in detoxification mechanisms that in turn incur growth and development costs (Kessler and Baldwin, 2002). Plant parts that are of high fitness value or that are under a high risk of attack may be best protected by constitutive defenses, whereas others may be better defended by induced responses (Wittstock and Gershenzon, 2002).

Induced Plant Defenses

Plants can employ 'active' or induced mechanisms in which the synthesis of defensive compounds is induced in response to insect attack (Harborne, 1988). Induced resistance itself has a fitness cost (Baldwin, 1998; Heil and Baldwin, 2002), but this cost is realized only if pest attack occurs, and can thus be less than that involved in constitutive defenses (Simms and Fritz, 1990). Typically, the induced defense response occurs both at the site of damage by the insect pest, and systemically throughout the plant.

Several signaling pathways are involved in the induction of defenses. Salicylic acid (SA) is crucial for local hypersensitive responses and systemic acquired resistance against many plant pathogens (Moran and Thompson, 2001). Resistance against herbivorous insects and some fungal pathogens depends on wound-response signaling via jasmonic acid (JA) and ethylene (Baldwin et al., 1997). Tissue damage caused by insect feeding activates an octadecanoid signaling cascade that ultimately produces JA (Creelman and Mullet, 1997). Mutations that reduce JA production result in increased susceptibility to herbivores. For example, an *Arabidopsis* triple mutant (*fad3-2 fad7-2 fad8*) lacks wound-induced JA biosynthesis, and as a consequence is more susceptible to fungal gnats (McConn et al., 1997). Resistance to fungal gnats was restored when JA was added to the mutant plants, demonstrating that JA is necessary for resistance.

In response to mechanical damage and insect attack, plants undergo a complex series of chemical and biochemical changes that can assist in the prevention of further tissue losses (Karban and Baldwin, 1997). This induced response often reduces the

nutritional quality of the tissues and may include the accumulation of proteinase inhibitors, phenolics, and alkaloids (Baldwin, 1988; Green and Ryan, 1972; Schultz and Baldwin, 1982). Herbivore attack is usually associated with wounding. Mechanical wounding alone induces the plant hormones ethylene and JA (Schmelz et al., 2003a). Plants frequently recognize herbivore attack through modification of the plant wounding response. Some plants use secondary metabolites that are specifically targeted against organ systems unique to herbivores.

The classic example of the plant wounding response is the synthesis of proteinase inhibitors in leaves of potato (*Solanum tuberosum*) or tomato (*Lycopersicon esculentum*) in response to feeding by larvae of lepidopteran pest species such as tobacco hornworm (*Manduca sexta*) (Green and Ryan, 1972). This system has been studied extensively to determine the role of JA and intermediates in both the local and systemic response to insect feeding. Tomato mutants deficient in wound- and systemin-induced JA accumulation are more susceptible to attack by the chewing insect *M. sexta* larvae (Howe et al., 1996; Lightner et al., 1993). Very similar results were found using these mutant plants and the cell content-feeding, two-spotted spider mite (*Tetranychus urticae* Koch) (Li et al., 2002). Li et al. (2002) also showed that transgenic tomato plants that constitutively activate the octadecanoid pathway (pathway for JA biosynthesis) were highly resistant to attack by spider mites and western flower thrips (*Frankliniella occidentalis*). Hence, the octadecanoid pathway appears to be essential for defense against both chewing and cell-content feeding herbivores in tomato.

Jasmonic Acid (JA)

Physical damage to cell walls (often caused by herbivores) initiates jasmonic acid (JA) biosynthesis (Creelman and Mullet, 1997). Wounding by herbivore feeding induces a systemic elicitor that interacts with plasma membrane receptors leading to the activation of lipase (Vick and Zimmerman, 1984). This results in the release of the fatty acid linolenic acid, which is a precursor for JA via the octadecanoid pathway. Allene oxide synthase (AOS) catalyzes the first JA specific reaction, and has been shown to be the major regulatory enzyme in the production of jasmonates (Laudert and Weiler, 1998). AOS catalyzes the conversion of linolenic acid to oxo-phytodienoic acid (OPDA), and this is necessary for the synthesis of defense proteins (Howe et al., 1996). Howe et al. (1996) used mutant tomato plants that were unable to convert linolenic acid to OPDA. The mutant plants were unable to induce certain defense proteins in response to insect (tobacco hornworm, *Manduca sexta*) attack and wounding, which compromised plant resistance toward the insect pest.

JA alters gene expression resulting in the accumulation of defense proteins such as proteinase inhibitors which affect the digestive system of attacking herbivores (Farmer and Ryan, 1992; Reinbothe et al., 1994) or in the accumulation of low molecular weight compounds with antibiotic properties (phytoalexins) (Blechert et al., 1995). Recently it was demonstrated in tomato, that two JA-inducible proteins (arginase and threonine deaminase) act in caterpillar (*Manduca sexta* larvae) midgut to catabolize the essential amino acids arginine and threonine, respectively (Chen et al., 2005). Their

results demonstrate that catabolism of amino acids in the insect digestive tract by host enzymes plays a role in plant protection against herbivores.

Creelman and Mullet (1997) provided insight into JA's dual role in plant development and defense. JA is involved in regulating genes that encode vegetative storage proteins (VSPs) and genes that encode proteins involved in plant defense in a similar fashion. Both types of genes are expressed in response to wounding and application of JA. Healthy plants accumulate VSPs in vacuoles that can be used in plant growth. Proteins involved in plant defense may be allocated to vacuoles for future use. It seems likely that proteins involved in plant defense may be used as VSPs, when defensive responses are not needed (Creelman and Mullet, 1997). It seems logical that instead of sequestering energy for separate processes, plants can use limited energy more efficiently by activating VSPs to induce defense responses.

Evidence for JA Inducing Volatile Emissions

Plants can also attract predators of insect herbivores by releasing volatile chemicals (i.e. indirect defense). Several studies have demonstrated a role of JA in plant volatile emission due to herbivory. Schmelz et al. (2001) used beet army worms and intact corn seedlings to examine the role of induced JA levels in volatile emission. They found a positive relationship between insect herbivore-induced JA levels and volatile emissions such as indole and the sesquiterpenes. The role of ethylene in this system was also examined. Plants treated with 1-methylcyclopropene (inhibitor of ethylene perception) exhibited no differences in JA levels, but had decreased volatile emissions

(Schmelz et al., 2003b). Ethylene and JA seem to act synergistically to induce production of volatiles in this system. JA and other signaling pathways can interact to induce pest-specific volatile emissions and/or stabile compounds used for defensive purposes.

Interestingly, in addition to attracting beneficial insects, it is postulated that plants wounded by herbivores communicate with neighboring, un-wounded plants via volatile emissions. Arimura et al. (2000) described the following mechanism of plant-plant interactions among lima bean plants that are infested with spider mites. In response to spider mites, a lima bean plant induces expression of at least six defense genes and emits volatiles accordingly. After plants are exposed to the terpenoids in the volatiles, a lypoxygenase (LOX) gene is induced in neighboring plants (Arimura et al., 2000). LOX in the octadecanoid pathway is a key enzyme in the biosynthesis of JA (Creelman and Mullet, 1997). LOX then induces the activation of the JA signaling pathway, and then JA mediates the expression of multiple defense genes. Arimura et al., (2000) postulate that neighboring plants can recognize the specificity of the volatiles and act accordingly. If so, these plants can prepare defenses against specific insect pests in advance.

Effects of Exogenous JA Applications

Exogenous treatment with JA may have applications in pest control management by affecting the pest, and also the natural enemies of the pest. Exposure to JA or methyl jasmonate reduced feeding of caterpillars in the laboratory (Avdiushko et al., 1997) and

also in the field (Baldwin, 1998; Thaler et al., 1996). Using cotton leaf discs, preference was reduced by more than 60% for aphids and spider mites, and more than 90% for thrips in JA-induced leaves compared with control leaves (Omer et al., 2001). JA is also known to increase the production of volatiles throughout the crop, independent of herbivore presence. Spider mite damage and JA treatment have similar, although not identical, effects on volatile induction in lima bean plants (Dicke et al., 1999), and gerbera daisy (Gols et al., 1999). JA treated plants have been shown to increase volatile emissions that attract natural enemies, thus increasing the mortality rate of particular insect pests in gerbera (Gols et al., 1999), maize (Ozawa et al., 2004), native tobacco (Halitschke et al., 2000; Kessler and Baldwin, 2001), tomato (Thaler, 1999), and rice (Lou et al., 2005). In addition to attracting natural enemies of the herbivores, the release of volatile organic compounds can function as a direct defense by repelling the ovipositing herbivores (De Moraes et al., 2001; Kessler and Baldwin, 2001). If using exogenous applications of JA to induce indirect defenses, JA should preferably be applied when taking into account the distribution of the pest, in order to avoid confusing the natural enemies (Gols et al., 1999).

Interactions Between JA and Ethylene

Insect herbivory is known to promote an increase in JA accumulation and ethylene emission (Arimura et al., 2002; Kahl et al., 2000; Schmelz et al., 2003a). In lima bean leaves, exogenous applications of ACC, the key precursor in the biosynthesis of ethylene, increases the activity of JA in the stimulation of volatile emission (Horiuchi

et al., 2001). Ethylene has also been proposed to enhance the wound-induced accumulation of JA in tomato, suggesting that induced JA levels may be partly ethylene dependent (O'Donnell et al., 1996). In most reported plant-herbivore interactions, ethylene and JA act synergistically to induce higher levels of genes encoding defense responses. For example, ethylene and JA have been shown to act synergistically in the expression of defensive genes in maize (*Zea mays*) and native tobacco (*Nicotiana tobacum*) (Schmelz et al., 2003c). However, in *Nicotiana attenuata*, herbivore-induced ethylene antagonizes JA-induced production of the defensive compound, nicotine (Kahl et al., 2000; Winz and Baldwin, 2001).

Elicitors from herbivores can cause herbivore-specific defensive responses. Any compound from an herbivore that interacts with plants on a cellular level is a possible elicitor. Elicitors from insect oral secretions have been isolated and used to study pest-specific responses. In maize, beet armyworm (*Spodoptera exigua*) herbivory was found to increase ethylene, whereas volicitin [N-(17-hydroxylinolenoyl)-L-Gln] treated plants showed no increase in ethylene (Schmelz et al., 2003b). Volicitin is a non-enzymatic elicitor of plant volatile emission isolated from beet armyworm oral secretions (Alborn et al., 1997). It appears that ethylene derived from insect damage interacts with volicitin and accumulated JA to result in increased levels of volatile emission (Schmelz et al., 2003b).

The response of tobacco plants (*Nicotiana attenuata*) to *Manduca* caterpillar feeding demonstrates a link from the elicitor to downstream signaling components (Halitschke et al., 2001). The elicitors involved in altering defense responses in this

system are comprised of fatty-acid-amino-acid conjugates (FACs). The tobacco plants respond to wounding with a burst in jasmonic acid. This response is amplified by caterpillar feeding and the application of FACs to wounds, but wounding alone did not induce production of volatiles that function as predator attractants in the plant's indirect defense (Halitschke et al., 2001). Jasmonic acid alters gene expression and induces the production of volatiles used as an indirect defense and the production of nicotine for direct defense in tobacco plants (Baldwin et al., 1997). Caterpillar feeding and subsequent oral secretions to wounds also induced an ethylene burst. Ethylene seems to be involved in regulating the production of nicotine in this system. This may be an adaptation to the specialist herbivore *Manduca*. The *Manduca* caterpillar is able to tolerate nicotine and possibly use it for its own defense (Kessler and Baldwin, 2002). Perhaps the plant recognizes the herbivore and induces the production of ethylene in order to regulate nicotine production in this case.

Much research is being conducted to determine the pathways involved in resistance to herbivores. Plant resistance is becoming an increasingly popular idea, especially in response to incentives for reduced pesticide use (Holtzer et al., 1996). However, insects and plants are always adapting to each other, and eventually insect pests develop resistance to defensive compounds produced by plants. Increasing resistance to one pest could increase susceptibility to other insect pests. The fitness costs to plants with increased resistance must always be considered because improving one aspect of plant development often reduces other plant qualities. Increased pest resistance would be most beneficial, when it does not compromise important plant characteristics.

Likely, both insect resistance and plant development characteristics must be compromised in order to produce economically feasible plants.

Effects of Fertilization on Host Plant Resistance

Effects of Fertilization on Secondary Metabolism and Insect Herbivores

The growth-differentiation balance hypothesis (GDBH), developed by Herms and Mattson (1992), is premised upon a physiological trade-off between growth and differentiation processes. Secondary metabolism and structural reinforcement are physiologically constrained in dividing and enlarging cells, and requires photosynthates that could be used for growth processes. Plants have limited resources to support their physiological processes, and all requirements cannot be met simultaneously. Hence, trade-offs occur among growth, storage, reproduction and defense (Lambers and Poorter, 1992).

Fertilization frequently decreases secondary metabolite concentrations in plants (Bryant et al., 1983; Koricheva et al., 1998; Kytö et al., 1996; Mattson, 1980; Waterman and Mole, 1989). The GDBH attributes this response to a resource-based physiological trade-off between primary and secondary metabolic pathways (Herms and Mattson, 1992). Resource availability is predicted to have a parabolic effect on constitutive secondary metabolism, with concentrations highest in moderately stressed plants, and lower in plants that are rapidly growing or severely carbon-stressed (Herms, 1999). Growth is more sensitive to stress than is photosynthesis, being reduced by even

moderate shortages of nutrients or water. Whereas, photosynthesis is not affected until stress becomes more severe (Körner, 1991, Luxmore, 1991). Hence, in summary, the GDBH predicts a positive correlation between growth and secondary metabolism at low to moderate levels of resource availability. At higher levels of resource availability photosynthesis is not affected, and negative correlation between growth and secondary metabolism is predicted (Herms, 1999; Herms and Mattson, 1992).

There is much evidence to support the GDBH, and it has proven useful in explaining physiological and ecological effects on phytochemical expression. Several studies on trees have been conducted to determine the effect of nutrient availability on secondary metabolites implicated in defense against herbivores (D. Herms, personal communication). These studies, and others, have shown that nutrient stress increases allocation to secondary metabolism (i.e. phenolics) in several tree species, and this increase in phenolics negatively affects the feeding of a variety of insect species (Hale et al., 2005; Keski-Saari and Julkunen-Tiitto, 2003; Lloyd et al., 2006).

The effects of resource availability on plant defensive compounds have been studied extensively. The quantity and quality of N has received the most attention in this regard. Low levels of N results in higher levels of phenolics in tomato (Stout et al., 1998; Wilkens et al., 1996). The increase in phenolics under conditions of low N is often described as a passive consequence of the increased flow of excess photosynthate into secondary metabolism (the C/N balance hypothesis) (Waterman and Mole, 1989). However, there is evidence that the increase in phenolics is actively controlled by the plant (Berenbaum, 1995). Nutrient stress in tomato increases the expression of several

genes involved in phenolic metabolism (Bongue-Bartelsman and Phillips, 1995). This is supported by the fact that young leaves in nitrogen-deficient plants have higher levels of phenolics compared to older leaves (Stout et al., 1998; Wilkens et al., 1996). Young leaves have higher levels of N, and would be expected to show lower allocation to secondary metabolism if the allocation was strictly passive. Stout et al. (1998) also showed that induction of proteinase inhibitors and polyphenol oxidase were not affected by nitrogen availability, however, polyphenol oxidase was constitutively higher in low N plants. Polyphenol oxidases are antinutritive enzymes that decrease the nutritive value of the wounded plant by cross-linking proteins or catalyzing the oxidation of phenolic compounds to reactive and polymerizing quinones (Kessler and Baldwin, 2002). Proteinase inhibitors and polyphenol oxidase are both nitrogen containing molecules, thus this is evidence for an active, damage-induced increase in allocation to defense that overrides physiological constraints (Stout et al., 1998).

Inbar et al. (2001) used a variety of growing conditions to test the effects on the suitability of the host plant (tomato) to various insect pests. There was a positive association between insect performance and plant growth, and there was a negative association between plant growth and chemical defense, which supports the GDBH. Plants subjected to nutrient stress had higher levels of total phenolics and peroxidase, which negatively affected the prolificacy of the insect pests (whitefly, leafminer, and corn earworm). Phenolics are the dominant allelochemicals in tomato (and many other plants) and known to decrease insect growth, development and survival (English-Loeb et al., 1997; Isman and Duffey, 1982; Stamp 1990; Wilkens et al., 1996).

The density of trichomes is a constitutive defense present in many plants that can negatively affect insect foraging. In wild tomato (*Lycopersicon hirsutum* f. *glabratum*), trichome- and lamellar-based resistance decreased with increasing fertilizer regime (Barbour et. al., 1991). The increased fertilization decreased both the amount of 2-tridecanone per trichome tip, and the density of glandular trichome which contain 2-tridecanone. 2-tridecanone is toxic to the tobacco hornworm (*Manduca sexta* L.) and Colorado potato beetle (*Leptinotarsa decemlineata* Say), which are pests of tomato. The decreased density of the trichomes appears to be a direct effect of NPK fertilizer, and not an indirect effect (e.g. a dilution effect due to increased foliar growth at higher fertilizer regimes), since leaf area remained constant or decreased slightly with increased fertilizer regime (Barbour et. al., 1991).

Effects of Fertilization on Jasmonic Acid and the Induced Response

There are two experiments that have documented the effect of N on the accumulation of JA and subsequent volatile emissions. In the first documented experiment, N deficiency resulted in elevated and prolonged increases in wound- and volicitin-induced JA levels in maize (Schmelz et al., 2003b). Low N plants displayed strong synergistic interactions between volicitin and ethylene, as indicated by increases in induced sesquiterpene and indole emissions. Ethylene addition did not increase JA and 1-MCP (compound used to suppress ethylene responses) pretreatment did not suppress JA levels. In maize seedlings, root sensitivity to ethylene and subsequent aerenchyma formation can be increased by 100-fold during periods of N-deficiency (He

et al., 1992), even though actual ethylene production is suppressed (Drew et al., 1989). In maize, N deficiency appears to influence volicitin-induced sesquiterpene emission by increasing the duration and magnitude of induced JA levels and by altering the interaction between ethylene and JA signals through changes in ethylene sensitivity (Schmelz et al., 2003b).

In contrast to maize, native tobacco (*Nicotiana attenuate*) treated with larvae oral secretions (OS) or water had reduced levels of JA accumulation in low N plants (Lou and Baldwin, 2004). JA levels were also constitutively lower in low N plants. The expressions of genes involved in JA biosynthesis (lypoxygenase 3 and allene oxide synthase) were more up-regulated in high N plants, which is consistent with endogenous JA levels. Previously, they showed that wounding plus water or OS, and methyl jasmonate (MeJA) increases nicotine levels locally and systemically in high nitrogen plants, but not in low N plants (Lou and Baldwin, 2003). Low N plants had higher constitutive nicotine levels than high N plants, but lower induced levels. *N. attenuata* must have a means of giving priority to the allocation of N to nicotine biosynthesis during N-limited growth. Three C-containing secondary metabolites (chlorogenic acid, rutin, and diterpene glycosides), were also higher in low N plants. In contrast to maize (Schmelz et al., 2003b), there were no differences in volatile organic compound (VOC) emissions between low N and high N plants (Lou and Baldwin, 2004).

Possible reasons for the ability of N-deficient plants to produce greater elicitor-induced defense responses include the fact that N deficiency commonly results in increased levels of leaf glucose, fructose, sucrose, and starch (Paul and Driscoll, 1997;

Rufty et al., 1988). In addition to providing an increased source of nonstructural carbohydrates to potentially fuel volatile biosynthesis, elevated levels of soluble sugars can enhance the expression of wound- and jasmonate-induced genes encoding proteinase inhibitors and VSPs (Johnson and Ryan, 1990; Mason et al., 1992). The influence of N availability on rapidly induced responses has been examined for furanocoumarins in parsnip (*Pastinaca sativa*) (Zangerl and Berenbaum, 1995) and protease inhibitors in tomato (Stout et. al., 1998). In both cases, N availability had little or no effect on the induced concentrations of these defenses. As previously noted, induced nicotine accumulation was attenuated in tobacco, but the constitutive level of nicotine in low N plants were higher than high N plants (Lou and Baldwin, 2004).

The availability of potassium (K+) also appears to play a role in JA biosynthesis. Armengaud et al. (2004) used full genome microarrays to assess transcriptional responses of *Arabidopsis* seedlings to changing external supply of potassium (K+). The most prominent response was found for genes linked to jasmonic acid. K-starvation caused a strong increase in transcript levels for the JA biosynthetic enzymes lipoxygenase, allene oxide synthase, and allene oxide cyclase, and the expression of these genes were rapidly decreased in response to K+ resupply. A large number of JA responsive genes were also up-regulated in response to K-starvation. Ashley et al. (2006) provided more evidence for K+ deficiencies showing similarities to those involved in stress responses to wounding and insect and pathogen attacks, in which JA and derivatives play a prominent role. By contrast, in nitrogen and phosphorus starved plants, no JA-responsive genes were identified by expression profiling of up to 8000

genes, although differential regulation of several defense-related transcripts was observed (Hammond et al., 2003; Wang et al., 2000; 2003; Wu et al., 2003). JA is known for its role in plant defense response against insect herbivores and (mainly necrotrophic) fungi, which are the most relevant enemies of K+-starved plants (Kessler and Baldwin, 2002). Perhaps, the observed increase in JA serves to protect K+-deficient plants against herbivore and fungal attack, while not entirely compensating for increased feeding and pathogen development.

Gerbera and Host Plant Resistance

Gerbera jamesonii Bolus ex Hooker f. is an economically important floriculture crop that is sold as cut flowers, bedding plants, or as a potted flowering plant. Floriculture crop sales were $5.4 billion in 2005 (Floriculture and Nursery Crops Yearbook, 2006). Gerbera daisies sold as cut flowers represented almost 1% of the U.S. sales for cut flowers, with over $32 million in sales for 2005. Unfortunately, gerberas make excellent hosts for insect pests, especially western flower thrips [(WFT) *Frankliniella occidentalis* Pergande]. Gerberas have even been suggested for use as a 'trap crop' for managing WFT (Blumthal et al., 2005).

Host plant resistance characteristics of gerbera have not been well-documented. Marcel Dicke's lab has reported several studies focused on the *Gerbera jamesonii*: two-spotted spider mite (*Tetranychus urticae* Koch) interaction. They showed that cultivars of gerbera differ in their suitability for spider mites (Krips et al., 1998), with one cultivar

('Bianca') exhibiting resistance compared to the other more susceptible cultivars. This host plant resistance to spider mites did not influence the capacity for population increase of the predatory mite *Phytoseiulus persimilis*; therefore, gerbera host plant resistance seems compatible with biological control for spider mites (Krips et al., 1999). They discovered that spider mites feeding on gerbera leaves induced a highly complex blend of volatiles which attract the predatory mite *P. persimilis* (Krips et al., 2001). Jasmonic acid applied to gerbera leaves resulted in a very similar volatile blend that also attracts the predator (Gols et al., 1999). In summary, gerberas differ largely in resistance to *T. urticae*, however, the mechanism of this resistance is not known. Secondly, gerberas are capable of producing volatile compounds in response to spider mite feeding and/or exogenous JA applications; and these volatile compounds aid in an indirect plant defense strategy, i.e. attraction of natural enemies of herbivores.

To my knowledge, there has been no evidence presented that denotes specific endogenous plant compounds present in gerbera that are used in direct defense against herbivores. Two glucoside metabolites, parasorboside and gerberin, are present in large amounts in *Gerbera hybrida* and may contribute to insect and pathogen resistance (Eckermann et al., 1998; Yrjönen et al., 2002), but to date, there is no documented evidence of this. There is also little documented research pertaining to the effects of fertilization on insect herbivores or plant defense characteristics of gerbera. Recently, however, it was reported that the density of eggs and nymphs of whitefly [*Trialeurodes vaporariorum* (West.)] on gerbera was higher in free choice assays and when nitrogen was supplied (Ortega-Arenas et al., 2006).

Western Flower Thrips

Thrips are cell-content-feeding insects that penetrate single cells with a stylet to suck out the contents (Kindt et al., 2003). Western flower thrips [(WFT) *Frankliniella occidentalis* Pergande] were known in New Zealand as early as 1934 (Mound and Walker, 1982), but they were restricted almost entirely to the flowers of *Lupinus* species. Presumably, the lupin strain was introduced from California along with its host plant long before the aggressive pest strain evolved (Mound, 2004). Global trade in ornamental greenhouse plants rapidly spread a highly pestilent polyphagous strain of *F. occidentalis*, a species native to the southwestern United States around the world beginning in the mid-1980s (Morse and Hoddle, 2006). Now it appears that there is little limit to the range of plants, and plant parts, attacked by WFT.

WFT are extremely opportunistic (Mound and Teulon, 1995), feed on a wide range of plant tissue types (Trichilo and Leigh, 1988) and can even be predacious (Gonzalez and Wilson, 1982; Trichilo and Leigh, 1986). WFT prefer to feed within flower buds and flowers, which also serve as protection from insecticides and predators. However, when they have the option, WFT feed on mite eggs instead of leaf tissue; and this reaction is enhanced if the leaves have been exposed previously to spider mite feeding (Agrawal et al., 1999). They may also supplement their diet by feeding on thrips larvae (Van Rijn et al., 1995). When WFT feed on expanding tissues, the tissue becomes distorted because the affected cells are unable to expand. WFT feeding damage on expanding leaf tissue looks ‘silver’ because the cells are full of air (De Jager

et al., 1995). In addition to direct or cosmetic plant damage, WFT vector the tospovirus, tomato spotted wilt virus.

In ornamental greenhouse crop production, there is a low threshold for insect pests, including WFT, because the aesthetic quality of the whole plant must be excellent. WFT are difficult to control because of their small size, ability to reproduce to high numbers, cryptic behavior, egg deposition inside plant tissue, and propensity to secrete themselves in tight spaces. Also, WFT appear to have a propensity for becoming resistant to insecticides. Many populations of WFT have shown resistance to a number of different insecticides, including insecticides with different chemical classes (Brøadsgaard, 1994; Espinosa et al., 2002; Immaraju et al., 1992; Zhao et al., 1995). This is partially due to the current reliance on insecticides for control of WFT. To avoid the development of insecticide resistance, producers need to reduce their reliance on insecticides and develop alternative management strategies. Ideally, an integrated pest management system that combines host plant resistance, cultural practices, biological control, and chemical control should be employed to control WFT in greenhouse production. Altering fertilization is a cultural practice that may facilitate in the control of WFT and reduce insecticide applications.

Effects of Fertilization on WFT

It has been well-documented that plant nutrient status has an effect on insect herbivory. Many insect herbivores are more prolific on plants treated with higher nitrogen fertility. The growth and reproduction of phytophagous insects is often limited

by the nutritional content of their hosts, and generally increases as foliar nitrogen content increases (Mattson 1980).

High fertility has been shown to increase the productivity of western flower thrips [(WFT) *Frankliniella occidentalis* (Pergande)], a major pest on both ornamental and agricultural plants. Fertilizer rates representing 0%, 10%, 20% and 100% the recommended rate of N applied to chrysanthemum resulted in an increase in WFT populations as fertilization rates increased (Davies et al., 2005). In tomato, the actual N content in the flowers correlated with WFT abundance (Brodbeck et al., 2001). In separate studies, applying fertilizers with higher than recommended rates of N resulted in an increase in WFT populations in chrysanthemum (Schuch et al., 1998) and tomato (Stavisky et al., 2002). In contrast, Chau and Heinz (2006) reported significantly lower levels of WFTs on chrysanthemums fertilized at ≤ 50% or ≥ 150% the recommended level. Chen et al. (2004) reported that supplying a luxurious amount of N to impatiens had no affect on WFT populations when compared to impatiens receiving an optimum level of N. However, a similar increase in phosphorus resulted in more adult WFT four weeks after inoculation – indicating that excess phosphorus accumulation, but not N, may support more rapid growth of WFT populations in impatiens. Whether supra-optimal levels of fertilization affect WFT populations may depend on the plant-fertilizer combination. Nonetheless, it does appear that lowering fertilization below recommended levels for chrysanthemum and tomato reduces the prolificacy of WFT.

The within plant distribution of WFT was observed in developing chrysanthemums grown under different fertilization regimes to ascertain whether WFT

would migrate towards areas of higher nitrogen content (Chau et al., 2005). WFT distribution did not correlate to N content of plant tissues, but to the phenology of the plant. WFT reportedly stayed in the middle regions of the plant until flowers began to open, at which time they migrated to flowers. The apical regions of plants typically contain higher N content, but they also may contain higher amounts of phenolics compared to older leaves (Stout et al., 1998; Wilkens et al., 1996). Perhaps the nutritional advantage in the young leaves is minor, and they are content to feed on more physiologically mature leaves until reproductive tissue (and eventually pollen) is available.

Pollen has comparatively high concentrations of nitrogen (Slansky and Scriber, 1985), and preference for this tissue is often cited as a means of obtaining a higher protein diet (Kirk, 1997). Pollen increases the rate of growth, development, and fecundity for WFT (Hulshof et al., 2003; Trichilo & Leigh, 1988; Ugine et al., 2006a), and it has been suggested that manipulating the abundance of this resource could have widespread implications for managing thrips populations (Ugine et al., 2006b). The effects of fertilization on aromatic amino acids may also play a role in WFT activity. Mollema and Cole (1996) found consistent relations between aromatic amino acid concentrations (phenylalanine and tyrosine) of foliar tissue and the degree of damage caused by WFT. Higher fertilization rates increased the concentration of phenylalanine in tomato flowers, and this increase was highly correlated with WFT abundance (Brodbeck et al., 2001). Thus, nitrogen form as well as total N concentration may be critical to the nutritional ecology of WFT.

Summary

Plants use constitutive and induced defenses to aid in protection against phytophagous insects. Nutrient availability has a substantial effect on both of these resistance mechanisms. When nutrient limitations reduce growth, allocation to secondary metabolism is favored, and total phenolic concentrations within the plant are typically increased. Jasmonates represent the best characterized class of signals mediating the elicitation of defense responses to wounding and herbivory, and they have been shown to be necessary for the induction of a wide array of defensive compounds. N deficiency has been shown to affect JA accumulation in contrasting ways: JA accumulation is increased in maize (Schmelz et al., 2003b) and decreased in tobacco (Lou and Baldwin, 2004). Apparently, these species have developed different mechanisms to cope with N deficiency due to their evolutionary history.

Nutrition of host plants has been shown to have a direct effect on the prolificacy of numerous insect pests, including WFT. Reduced fertilization decreased WFT abundance in chrysanthemum (Chau et al., 2005; Chau and Heinz, 2006; Davies et al., 2005; Schuch et al., 1998) and tomato (Stavisky et al., 2002), and this effect has been attributed to the reduced availability of essential nutrients for WFT, and possibly secondary metabolites. However, the effects of fertilization on constitutive defenses (e.g. secondary metabolites) and induced defenses (e.g. JA accumulation) have not been measured in any of these studies. The effect of fertilization on essential elements of the WFT diet do not account for all of the observed results. There is limited information on

the effects of nutrient availability on JA accumulation –just two studies that reported contradictory effects of N deficiency on JA. It is important that we determine the effects of fertilization on JA accumulation in other plant species in order to ascertain whether altering fertilization is a possible mechanism for manipulating induced host plant resistance, as well as constitutive plant defense.

There is wide interest in implementing best management practices (BMPs) in greenhouse crop production. The emphasis is on reducing inputs such as fertilization (and subsequent fertilizer run-off), water (due to the growing water shortages), and pesticides. In order to optimize fertilization, it will be beneficial to know the influence of fertilization on host plant quality, host plant resistance and pest populations. With this knowledge, fertilization recommendations can be made that maintain high crop quality, while minimizing insect damage and the need for intensive pesticide use, with associated chemical phytotoxicity (Spiers et al., 2006).

The floriculture crop *Gerbera jamesonii* is an economically important crop with substantial nutritional requirements. Gerbera is also highly susceptible to a variety of insect pests, of which WFT are the most problematic. Hence, pesticide use on gerbera is prevalent. A goal of this research is to determine possible effects of fertilization on constitutive defense (i.e. phenolics) and induced defense (i.e. JA) mechanisms present in gerbera. In order to ascertain the practicality of altering fertilization to increase host plant resistance, the effects of fertilization on WFT abundance and feeding, and the subsequent effects on plant quality, physiology, growth and development will be determined.

CHAPTER III

FERTILIZATION AFFECTS CONSTITUTIVE AND WOUND-INDUCED CHEMICAL DEFENSES IN *Gerbera jamesonii*

Plants use constitutive and induced defenses to protect themselves from herbivore attack. Constitutive defenses include stored allelochemicals that can reduce the attractiveness of plants to herbivores. Many of these metabolites can decrease tissue digestibility and/or increase toxicity to herbivores, and influence herbivore feeding, oviposition, growth and development, fecundity, and/or fertility (Walling, 2000). These natural secondary metabolites are major determinants of plant resistance to herbivores (Berenbaum, 1995). While toxic compounds (e.g., alkaloids, terpenoids, phenolics) poison generalist herbivores, specialists are forced to invest resources in detoxification mechanisms that in turn incur growth and development costs (Kessler and Baldwin, 2002). Plant parts that are of high fitness value or that are under a high risk of attack may be best protected by constitutive defenses, whereas others may be better defended by induced responses (Wittstock and Gershenzon, 2002).

The growth-differentiation balance hypothesis (GDBH), described by Herms and Mattson (1992), is premised upon a physiological trade-off between growth and differentiation processes. Secondary metabolism is physiologically constrained in dividing and enlarging cells, and requires photosynthates that could be used for growth processes. Plants have limited resources to support their physiological processes, and all

requirements cannot be met simultaneously. Hence, trade-offs occur among growth, storage, reproduction and defense (Lambers and Poorter 1992).

Fertilization frequently decreases secondary metabolite concentrations in plants (Bryant et al., 1983; Koricheva et al., 1998; Kytö et al., 1996; Mattson, 1980; Waterman and Mole, 1989). The GDBH attributes this response to a resource-based physiological trade-off between primary and secondary metabolic pathways (Herms and Mattson, 1992). The quantity and quality of nitrogen available to the plant has been shown to influence the constitutive levels of a wide variety of types of secondary metabolites, including glucosinolates (Hugentobler and Renwick, 1995), cardenolides (Hugentobler and Renwick, 1995), phenolics (Inbar et al., 2001; Stout et al., 1998; Wilkens et al., 1996), alkaloids (Baldwin et al., 1993), and furanocoumarins (Zangerl and Berenbaum, 1995). Tomato plants treated with low nitrogen had twice the total phenolic content as compared to high N plants with the highest levels of phenolics in young leaves (Stout et al., 1998). In a separate study, increases in defensive compounds (peroxidase and total phenolics) due to N deficiency was shown to negatively affect feeding and oviposition of various insect herbivores in tomato (Inbar et al., 2001).

In response to mechanical damage and insect attack, plants undergo a complex series of chemical and biochemical changes that can assist in the prevention of further tissue losses (Karban and Baldwin, 1997). This induced response often reduces the nutritional quality of the tissues and may include the accumulation of proteinase inhibitors, phenolics, and alkaloids (Baldwin, 1988; Green and Ryan, 1972; Schultz and Baldwin, 1982). Induced resistance itself has a fitness cost (Baldwin, 1998; Heil and

Baldwin, 2002), but this cost is realized only if pest attack occurs, and can thus be less than that involved in constitutive defenses (Simms and Fritz, 1990). Typically, the induced defense response occurs both at the site of damage by the insect pest, and systemically throughout the plant.

Physical damage to cell walls (often caused by herbivores) initiates JA biosynthesis (Creelman and Mullet, 1997). Wounding by herbivore feeding induces a systemic elicitor that interacts with plasma membrane receptors leading to the activation of lipase (Vick and Zimmerman, 1984). This results in the release of the fatty acid linolenic acid, which is a precursor for JA via the octadecanoid pathway. Allene oxide synthase (AOS) catalyzes the first JA specific reaction, and has been shown to be the major regulatory enzyme in the production of jasmonates (Laudert and Weiler, 1998). AOS catalyzes the conversion of linolenic acid to oxo-phytodienoic acid (OPDA), and this is necessary for the synthesis of defense proteins (Howe et al., 1996). Howe et al. (1996) used mutant tomato plants that were unable to convert linolenic acid to OPDA. The mutant plants were unable to induce certain defense proteins in response to insect attack.

The classic example of the plant wounding response is the synthesis of proteinase inhibitors in leaves of potato (*Solanum tuberosum*) or tomato (*Lycopersicon esculentum*) in response to feeding by larvae of lepidopteran pest species such as tobacco hornworm (*Manduca sexta*) (Green and Ryan, 1972). This system has been studied extensively to determine the role of JA and intermediates in both the local and systemic response to insect feeding. Tomato mutants deficient in wound- and systemin-induced JA

accumulation are more susceptible to attack by the chewing insect *M. sexta* larvae (Howe et al., 1996; Lightner et al., 1993). Very similar results were found using these mutant plants and the cell content-feeding two-spotted spider mite (*Tetranychus urticae* Koch) (Li et al., 2002). Li et al. (2002) also showed that transgenic tomato plants that constitutively activate the octadecanoid pathway (pathway for JA biosynthesis) were highly resistant to attack by spider mites and western flower thrips (*Frankliniella occidentalis*). Hence, the octadecanoid pathway appears to be essential for defense against both chewing and cell-content feeding herbivore.

JA alters gene expression resulting in the accumulation of defense proteins such as proteinase inhibitors which affect the digestive system of attacking herbivores (Farmer and Ryan, 1992; Reinbothe et al., 1994) or in the accumulation of low molecular weight compounds with antibiotic properties (phytoalexins) (Blechert et al., 1995). Recently it was demonstrated in tomato, that two JA-inducible proteins (arginase and threonine deaminase) act in caterpillar (*Manduca sexta* larvae) midgut to catabolize the essential amino acids arginine and threonine, respectively (Chen et al., 2005). Their results demonstrate that catabolism of amino acids in the insect digestive tract by host enzymes plays a role in plant protection against herbivores.

Recently, nitrogen deficiency was shown to increase volicitin-induced volatile emission and jasmonic acid accumulation in maize (Schmelz et al., 2003b). Volicitin, N-(17-hydroxylinolenoyl)-L-Gln, is a nonenzymatic elicitor of plant volatile emission identified from beet armyworm (*Spodoptera exigua*) oral secretions (Alborn et al., 1997). N deficiency resulted in elevated and prolonged increases in wound- and

volicitin-induced JA levels in maize (Schmelz et al., 2003b). However, the opposite was found in the native tobacco, *Nicotiana attenuata*. Both constitutive and induced JA levels are lower in low nitrogen *N. attenuata* plants as compared to high nitrogen plants (Lou and Baldwin, 2004). Also contrary to maize, N supply does not influence the elicited release of volatiles in *N. attenuata*. Apparently, these species have developed different mechanisms to cope with N deficiency due to their evolutionary history. To my knowledge, these are the only two species that the effect of N supply on JA accumulation has been determined. It would be of interest to determine the trend with a commercial floriculture crop.

Gerbera jamesonii (gerbera) is an economically important ornamental crop that is sold as a bedding plant, cut flower, and/or flowering potted plant. Gerberas are highly susceptible to insect herbivores and unlike agronomic or other field crops, there is near zero tolerance for insect pests in greenhouse crop production of gerbera. The fact that it is also used as a cut flower allows for more lenient insect pest control in vegetative stages. To my knowledge, there has been no evidence presented that denotes specific endogenous plant compounds present in gerbera that are used in direct defense against herbivores. Two glucoside metabolites, parasorboside and gerberin, are present in large amounts in *Gerbera hybrida* and may contribute to insect and pathogen resistance (Eckermann et al., 1998; Yrjönen et al., 2002), but to date, there is no documented evidence of this. There is also little, to no documented research pertaining to the effects of fertilization on insect herbivores or plant defense characteristics of gerbera.

Nutrition of host plants has been shown to have a direct effect on the fecundity of numerous insect pests, including western flower thrips [(WFT) *Frankliniella occidentalis* Pergande], which are often the most problematic insect pest in greenhouse gerbera production. Reduced fertilization decreased WFT abundance in chrysanthemum (Chau et al., 2005; Chau and Heinz, 2006; Davies et al., 2005; Schuch et al., 1998) and tomato (Stavisky et al., 2002), and this effect has been attributed to the reduced availability of essential nutrients for WFT, and possibly secondary metabolites. However, the effects of plant nutrition on constitutive defenses (e.g. secondary metabolites) and induced defenses (e.g. JA accumulation) have not been measured in any of these studies. The effect of fertilization on essential elements of the WFT diet do not account for all of the observed results. There is limited information on the effects of nutrient availability on JA accumulation, including the two studies that reported contradictory effects of N deficiency on JA. It is important to determine the effects of nutrition on JA accumulation in other plant species in order to ascertain whether altering fertilization is a possible mechanism for manipulating induced host plant resistance, as well as constitutive plant defense. The objective of this research was to determine possible effects of fertilization on constitutive defense (i.e. phenolics) and induced defense (i.e. JA) mechanisms present in the economically important greenhouse floriculture crop, *Gerbera jamesonii*.

Materials and Methods

Plant Cultural Conditions

Sixty rooted *Gerbera jamesonii* 'Festival Salmon' seedlings (Knox Nursery Inc., Winter Garden, FL) were transplanted into 6-inch standard pots (15.5 × 11.5 cm; 2050 cm^3) on 10 June 2005, and watered with de-ionized water. Sunshine Mix #1 (Sun Gro Horticulture Inc., Pine Bluff, AK) was used as potting media. Plants were grown in growth chambers at 24°C day/20°C night, 60% RH under a 14 h photoperiod. Light intensity was approximately 300 μmol s^{-1} m^{-2}. Plants were supplied with either 0X [only supplied with initial fertilizer charge present in professional growing media – which is a 5-6-12 (elemental analysis: 5N-2.6P-10K) fertilizer incorporated at 2.7 lbs yd^{-3} (1.6 kg m^{-3}) of mix], or 1X (200 mg L^{-1} N, in addition to fertilizer charge) the recommended fertilization. Constant feed (fertigation) is recommended for gerbera. Plants were fertilized with a 200 ml solution of Peters Professional Peat-lite special 15-16-17 (elemental analysis: 15N-7P-14.2K; Scotts-Sierra Horticultural Products Co., Marysville, OH) at 0 or 200 mg L^{-1} N at each watering (as needed).

Powdery mildew was evident on several plants during the 3rd week of production. Therefore, plants were sprayed with the fungicide, propiconazole (Banner Maxx®, Syngenta Crop Protection, Inc., Greensboro, NC) at the rate of 6 fl. oz. per 100 gal 22 d after transplanting. The experiment was terminated 29 d after transplanting on 8 July 2005 and total phenolic accumulation was determined.

The study was repeated on 9 September 2006, to ascertain the effects on jasmonic acid (JA) accumulation. Plant cultural conditions were the same as above. Propiconazole (Banner Maxx®) was also applied in the 3rd week of production to protect against powdery mildew. The second study was terminated 35 d (18 October 2006) after transplanting, for JA quantification.

Jasmonic Acid Quantification

All treatments, including an additional set of "time zero" undamaged controls (n=5) were initiated (9:00 am) and then harvested 0, 0.5, 1, 3, or 10 h later on day 35. Treatments consisted of ± mechanical wounding with a hemostat to one physiologically mature leaf from each plant, which was harvested at specified time intervals for JA quantification. Each mechanical wounding consisted of clamping (mashing) down with the hemostat to a set point, damaging approximately 90 mm^2 of leaf tissue. The sampled leaf was wounded with the hemostat on both sides of the midrib in the middle of the leaf, between the veins. Tissue samples for JA were frozen in liquid N_2 and stored at –80ºC before analysis. JA was measured using isotope dilution based gas chromatography-mass spectrometry.

Extraction Procedure

Frozen tissue samples were ground to a fine powder in liquid nitrogen with mortar and pestle. A 'mastermix' was prepared that contained 5 mL of methanol and 50 ng of 1,3-[^{13}C]-JA internal standard (Creelman and Mullet, 1995) for each sample. The extraction 'mastermix' was divided into 15 mL Corning extraction tubes, warmed to

50°C, and kept warm in a beaker of water. Frozen tissue samples were weighed (1 - 2.5 grams) and placed in the extraction tubes, quickly mixed, and returned to the 50 °C water bath. Tubes were periodically shaken and returned to the water bath for 15 minutes. Then tubes were centrifuged for 5 minutes at 4000 rpm. The supernatant was carefully removed and dried. The extraction process was repeated until the supernatants and pellets were colorless.

Sample Purification

Samples were initially purified by C_{18} solid phase extraction [C_{18} Bakerbond spe™ 3 mL extraction columns; packed with reversed phase Octadecylsilane (500 mg); J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ]. Columns were prepared by adding 3 mL of 80% methanol, and then preconditioned with 6 mL of 0.4% acetic acid. Samples were re-suspended in 2 mL of 0.4% acetic acid and poured through the column 3× (6 mL/sample). The column was washed 3× (6 mL) with same 0.4% acetic acid. Samples were eluted with 5 mL of 80% methanol and the eluate was dried.

Dried samples were re-suspended in 220 µL of filtered MeOH:HOAc (0.1 N) 35:65. Extracts were filtered through 0.2-µM syringe filters and 190 µL was applied to a C_{18} HPLC column (Alltech® alphaBond C_{18}, 300 x 3.9 mm, 125A, 10µ). Constituents were separated by HPLC on a linear gradient from 35% to 85% methanol in 0.1 N acetic acid, at 0.8 mL per minute. Fractions were collected based on elution times previously determined with authentic JA standards (23.2 to 24.8 min), and then dried.

Gas Chromatography-Mass Spectrometry

The dried fractions were re-dissolved in 100 μL of MeOH, transferred to labeled reacti-vials, and dried under N_2. Samples were then methylated by adding 50 μL of ethereal diazomethane, capping reacti-vials tightly, and vortexing. The capped vials were then allowed to rest in a shaded location under a fume hood for 20 minutes. After 20 minutes, the caps were removed and diazomethane was allowed to passively evaporate under the fume hood, until dry. The methylation procedure was then repeated. JA levels were determined by injecting samples into a Varian 3400 gas chromatograph equipped with a Saturn® 3 mass spectrometer. The sample was suspended in 15 μL of ethyl acetate and 5 μL was injected with a septum programmable injector (SPI) set at 220 °C. The column used was a DB-5 (30 m × 0.25 mm × 0.25 μm) with a temperature program of 60 °C to 250 °C at a rate of 15 °C min^{-1} (12.66 min), with helium as the carrier gas (constant flow rate 1 ml/min). The analytes were ionized by electron impact. JA detections were confirmed by matching the retention time (R.T. ~ 10.02 min) and mass spectrum of JA standards to the unconfirmed analyte. Endogenous JA levels were determined by comparing ^{12}C JA peak areas (*m/z* 224) to the ^{13}C JA internal standard peak areas (*m/z* 226).

Total Phenolics Quantification

Juvenile and physiologically mature leaves were harvested from the 0 and 10 h time interval plants for total phenolic measurements. Total phenolics were extracted from the 0 and 10 hour tissue samples to assess the constitutive and induced levels,

respectively. Total phenolic content of leaf tissue was evaluated based on a method adapted from Swain and Hillis (1959), which describes the Folin-Ciocalteau reagent assay utilizing chlorogenic acid for a standard curve. In brief, fresh leaf tissue was weighed and ground with mortar and pestle in 80% methanol (6 mL). Extracts were centrifuged at 14,000 rpm for 15 minutes and kept in freezer (-80ºC) until analysis. The reaction mixture consisted of mixing 30 µL of the extract with 90 µL of Na_2CO_3 and 150 µL of Folin-Ciocalteau reagent in a 96-well microplate. After 30 min the absorbance was measured at 725 nm using a KC-4 spectrophotometer (Biotek® Instruments, Inc. Winooski, VT). Results were expressed as milligrams of chlorogenic acid equivalents per gram of fresh weight tissue.

Plant Growth

To characterize plant growth, the dry mass (DM) of shoot tissue was measured at the termination of the experiment.

Macro- and Micro-Nutrient Analysis

In both experiments, additional plants at low and high fertility were used to determine nutrient content of leaf tissue. Physiologically mature leaves were harvested from each fertility treatment at the termination of the experiment (n=3). The mineral status of plants was then determined on a DM basis. The tissue samples were dried in a convection oven at 70ºC for 120 h. Dry leaves were ground in a Wiley mill. Tissue samples were sent to a commercial lab for nutrient analysis (J.R. Peters/Scott's Testing

Laboratory, Allentown, PA). Nitrogen content was determined using the Kjeldahl procedure (Rund, 1984). The remaining samples were digested in wet acid (Jones et al., 1991) and macro and micro-element determinations were made on an inductively coupled plasma atomic emission spectrophotometer (3510 ICP) using the procedures of Munter and Grande (1981).

Experimental Design

The experiments were arranged as a 2 fertility × 2 ± wounding × 5 time period factorial for JA accumulation. Each pot was a single replicate. There were three replications (n=3) arranged in a completely randomized design. Data were analyzed using analysis of variance (ANOVA; SAS Institute Inc., 2000), with fertility, wounding, and time period as main effects. For total phenolic concentration, the experiment was arranged as a 2 fertility × 2 (± wounding) × 2 (0 and 10 h time interval) × two (leaf positions: young and physiologically mature) factorial using ANOVA (n=3). For nutrient analysis, the experiment was designed to test the two fertility levels (n=3) using ANOVA.

Results

Effects of Fertilization on Jasmonic Acid

The effect of fertilization on shoot dry mass was highly significant ($P < 0.0001$). The shoot dry mass of gerberas receiving 1X fertilization (2.8 g) was approximately 2.2-

fold that of plants receiving 0X fertilization (1.2 g) (Fig. 3.1). Plants receiving 0X fertility had substantially lower N, P, K, and Zn compared to 1X fertility plants (Table 3.1). Based on general recommendations for high-quality gerbera tissue nutrient levels, the 0X plants were sufficient for potassium, but deficient in nitrogen and phosphorus. Wound-induced JA accumulation was significantly affected by fertilization ($P \leq 0.05$) (Fig. 3.2). In response to mechanical damage, JA accumulation increased more rapidly and the accumulation was sustained longer in 0X plants compared to 1X plants. In 0X plants, JA levels peaked 0.5 h after wounding, and JA accumulation was significantly higher 0.5 h and 3 h after wounding, compared to 1X plants (Fig. 3.2). JA accumulation peaked 1 h after wounding in 1X plants, but this level was not significantly higher than 0X plants. Ten hours after wounding, JA concentrations were not different among 0X and 1X plants, though levels were still higher than constitutive levels. There were no differences in constitutive levels of JA between 0X and 1X plants, and constitutive levels did not differ based on time samples were taken.

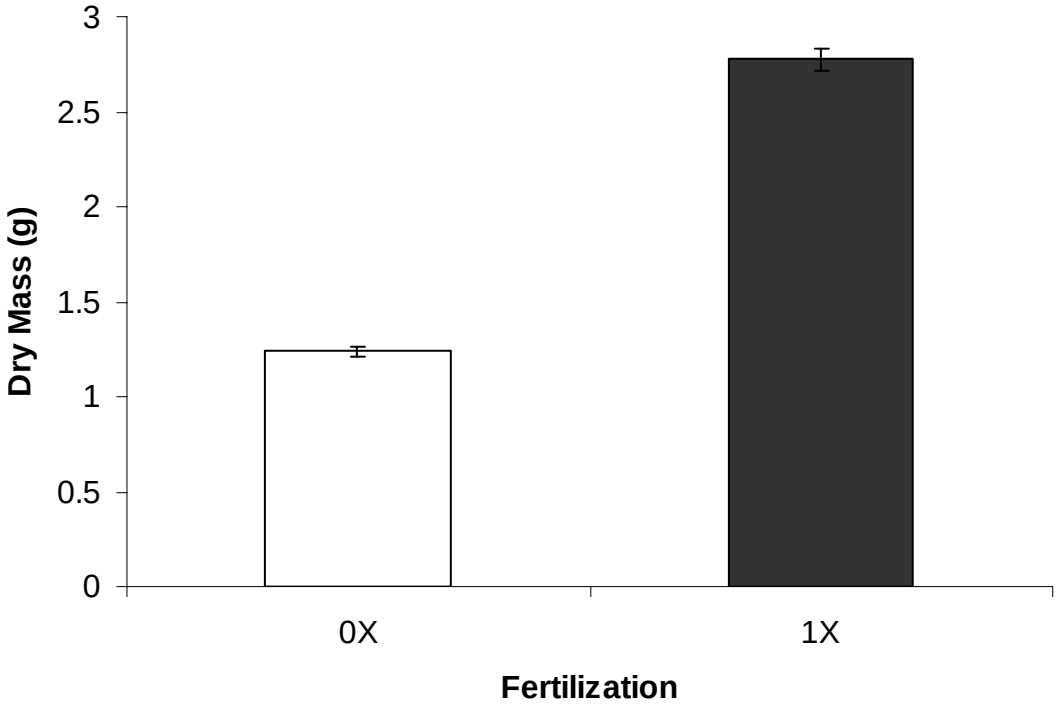

Fig. 3.1. Shoot dry mass of *Gerbera jamesonii* 'Festival Salmon' plants fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X) or 200 (1X) mg L^{-1} N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$); ± SE, n = 30.

Table 3.1. Effect of fertilization application on leaf macro- and micro-nutrient content of *Gerbera jamesonii* 'Festival Salmon'. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X) or 200 (1X) mg L^{-1} N at each watering, as needed; n = 4. The recommended levels are general recommendations for high-quality gerbera greenhouse crops (Dole and Wilkins, 1999).

	Macro-nutrients					Micro-nutrients							
Fertilizer	N	P	K	Ca	Mg	B	Fe	Mn	Cu	Zn	Mo	Al	Na
	%	%	%	%	%	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}
1X	4.1 ± 0.1	1.1 ± 0.0	4.3 ± 0.1	1.8 ± 0.0	0.6 ± 0.0	70.2 ± 2.6	76.1 ± 9.3	110 ± 4.8	5.2 ± 0.3	80.1 ± 1.9	0.3 ± 0.1	13.3 ± 1.6	486 ± 75.4
0X	1.1 ± 0.1	0.2 ± 0.0	3.8 ± 0.1	1.8 ± 0.1	0.7 ± 0.0	63.9 ± 4.4	55.1 ± 0.6	128 ± 7.5	8.3 ± 0.9	42.0 ± 3.9	0.3 ± 0.1	25.4 ± 3.2	436 ± 47.6
Recom-mended	2.7 – 4.1	0.3 – 0.7	3.1 – 3.9	0.4 – 4.2	0.3 – 2.8	19 – 50	60 – 130	30 – 260	2 – 10	19 – 80	---	---	---
Fertilizer	< 0.0001	< 0.0001	0.0099	NS	NS	NS	NS	NS	0.0272	0.001	NS	0.0272	NS

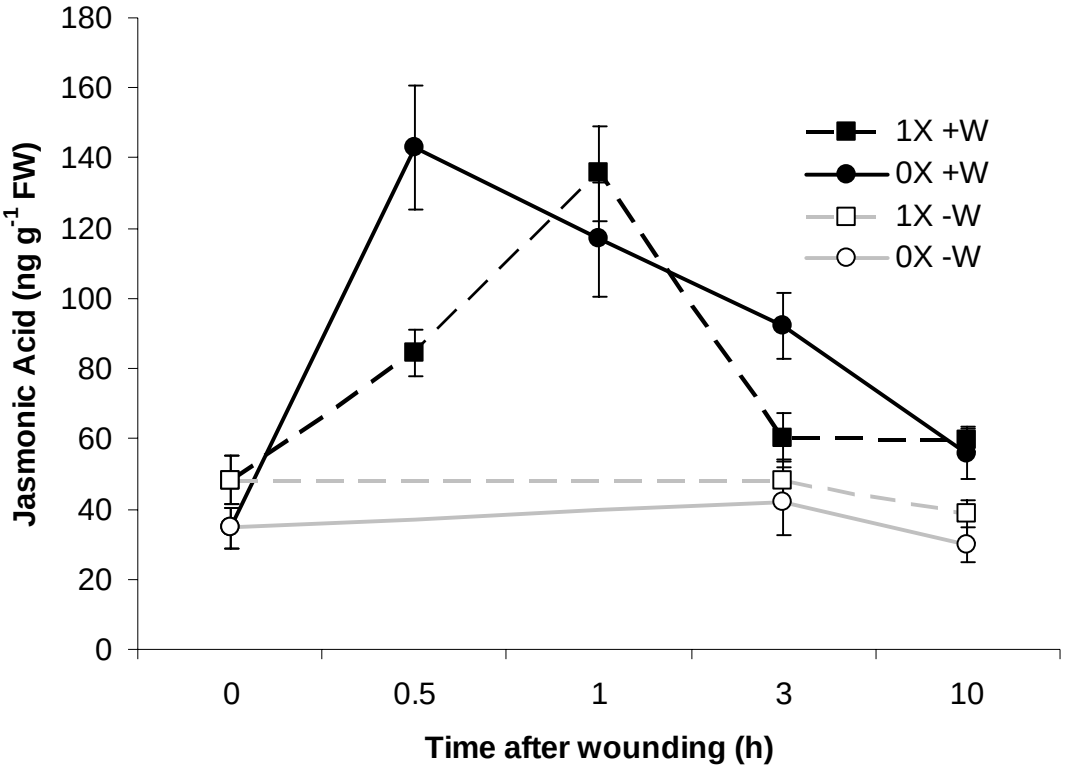

Fig. 3.2. Jasmonic acid (JA) accumulation in physiologically mature leaves from *Gerbera jamesonii* 'Festival Salmon' treated with two different fertilizer (F) concentrations and ± wounding (W). Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X) or 200 (1X) mg L^{-1} N at each watering, as needed. Treatments consisted of ± mechanical wounding with a hemostat to one physiologically mature leaf, which was harvested 0, 0.5, 1, 3, or 10h later for JA accumulation. Time (T) ($P < 0.0001$), W ($P \leq 0.0014$), and the interactions: F × W ($P \leq 0.054$), F × T ($P \leq 0.0041$), and F × T × W ($P \leq 0.0067$) were significant. Fertilization (F) and the interaction T × W were nonsignificant; ± SE, n = 5.

Effects of Fertilization on Total Phenolics

Fertilization had a substantial effect on the total phenolic concentration in gerbera leaf tissue ($P < 0.0001$). Physiologically mature leaves of 0X plants had approximately a 10-fold higher concentration of total phenolics, when compared to 1X

plants (Fig. 3.3). Leaf age also significantly affected the concentration of phenolics ($P \leq 0.0003$), with young leaves having greater concentrations of phenolics compared to physiologically mature leaves. Plants receiving 0X fertility had 13.0 mg of total phenolics g^{-1} fresh weight in young leaves compared to 7.2 mg of total phenolics g^{-1} fresh weight in physiologically mature leaves. Plants receiving 1X fertility had 1.9 mg of total phenolics g^{-1} fresh weight in young leaves compared to 0.8 mg of total phenolics g^{-1} fresh weight in physiologically mature leaves. The interaction between fertilization and leaf age was also highly significant ($P \leq 0.0089$). There were no differences in total phenolics due to wounding (data not shown).

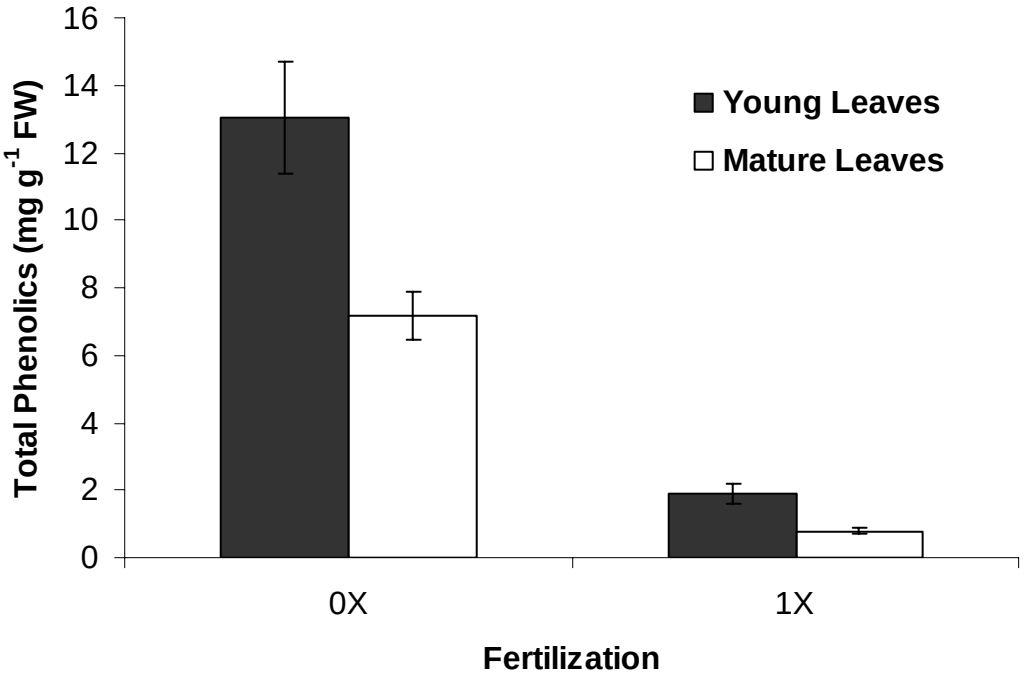

Fig. 3.3. The total soluble phenolic concentration in young and physiologically mature leaf tissue from *Gerbera jamesonii* 'Festival Salmon' fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X) or 200 (1X) mg L^{-1} N at each watering, as needed. Fertilization ($P < 0.0001$), leaf age ($P \leq 0.0003$) and the interaction among fertilization and leaf age ($P \leq 0.0089$) were significant; ± SE, n = 12.

Discussion

Previous studies have demonstrated that nutrient availability affects the productivity of phytophagous insects on host plants. This is the first study to report jasmonic acid (JA) accumulation in gerbera—detailing the influence of nutrient availability on endogenous chemical defenses present in gerbera. Reduced nutrient availability, predominantly nitrogen availability, has been shown to increase secondary metabolites (e.g. phenolics) that are implicated in defense against insect pests (Herms and Mattson, 1992; Inbar et al., 2001; Stout et al., 1998). In addition, nitrogen availability reportedly affects JA accumulation—which is the phytohormone predominantly responsible for induced host plant resistance in response to insect feeding damage. Interestingly, low N availability reportedly increases wound-induced JA accumulation in maize (Schmelz et al., 2003b) and decreases wound-induced JA accumulation in native tobacco (Lou and Baldwin, 2004). These contrasting results prompted us to investigate the effects of nutrient availability on JA accumulation, as well as the effects on constitutive (i.e. phenolics) chemical defenses in the commercial floriculture crop *Gerbera jamesonii*.

Based on our results, reducing fertilization in potted gerbera production may increase both constitutive and induced host plant resistance. Total phenolics were much higher in nutrient stressed plants (0X plants), demonstrating that allocation to secondary metabolism increases when nutrients are limiting. Wounding induced the rapid accumulation of JA, but there were no differences in total phenolics due to wounding.

Plants receiving 0X fertility increased wound-induced JA accumulation more rapidly and sustained this increase longer than plants fertilized with recommended rates (1X plants). This is evidence for an active, damage-induced increase in allocation to defense that overrides nutritional demands imposed by growth.

The effects of resource availability on plant defensive compounds have been studied extensively. The quantity and quality of nitrogen (N) has received the most attention in this regard. Low levels of N result in higher levels of phenolics in tomato (Stout et al., 1998; Wilkens et al., 1996). The increase in phenolics under conditions of low N is often described as a passive consequence of the increased flow of excess photosynthate in to secondary metabolism (the C/N balance hypothesis) (Waterman and Mole, 1989). However, there is evidence that the increase in phenolics is actively controlled by the plant (Berenbaum, 1995). Nutrient stress in tomato increases the expression of several genes involved in phenolic metabolism (Bongue-Bartelsman and Phillips, 1995). In our study, young leaves had higher levels of phenolics compared to older leaves—which parallels results found in nitrogen-deficient tomato plants (Stout et al., 1998; Wilkens et al., 1996). Young leaves have higher levels of N, and would be expected to show lower allocation to secondary metabolism if the allocation was strictly passive. Stout et al. (1998) also showed that induction of proteinase inhibitors and polyphenol oxidase were not affected by nitrogen availability, however, polyphenol oxidase was constitutively higher in low N plants. Polyphenol oxidases are antinutritive enzymes that decrease the nutritive value of the wounded plant by cross-linking proteins or catalyzing the oxidation of phenolic compounds to reactive and polymerizing

quinones (Kessler and Baldwin, 2002). Proteinase inhibitors and polyphenol oxidase are both nitrogen containing molecules, indicating that the wound-induced increase in allocation to defense overrides physiological constraints (Stout et al., 1998).

Phenolics are the dominant allelochemicals in many plants and are known to decrease insect growth, development and survival (English-Loeb et al., 1997; Isman and Duffey, 1982; Stamp 1990; Wilkens et al., 1996). As in our study, Inbar et al. (2001) reported a negative association between plant growth and chemical defense. They reported a positive association between insect performance and plant growth. Plants subjected to nutrient stress had higher levels of total phenolics and peroxidase, which negatively affected the prolificacy of insect pests (whitefly, leafminer, and corn earworm) (Inbar et al., 2001).

As previously noted, two reports have documented the effect of N on the accumulation of JA and subsequent volatile emissions. In the first report, N deficiency resulted in elevated and prolonged increases in wound- and elicitor-induced JA levels in maize (Schmelz et al., 2003b). In contrast to maize, native tobacco (*Nicotiana attenuate*) had reduced levels of wound- and elicitor-induced JA accumulation in low N plants (Lou and Baldwin, 2004). JA levels were also constitutively lower in low N tobacco plants. Our results are closer to those reported by Schmelz et al. (2003b). In maize seedlings, JA accumulation was similar for medium and low N plants between the time points of 0.5- and 2-h after mechanical wounding. However, in contrast to the short-term similarities, wound-induced JA accumulation was greater in low N plants between 4 and 12 h (Schmelz et al., 2003b). In our study with gerbera, JA was

significantly higher in 0X fertility plants 0.5- and 3-h after wounding; indicating that 0X gerbera plants accumulate JA more rapidly and sustain this increase longer than 1X plants. However, in contrast to maize seedlings, JA accumulation was not different between 0X and 1X plants 10 h after wounding. Interestingly, in both of the above-mentioned studies, JA accumulation appeared to peak 0.5 h after wounding for both high N and low N plants. In our study with gerbera, JA accumulated slower in 1X plants, and peaked at 1 h compared to 0.5 h after wounding in 0X plants.

It should be noted that direct comparisons cannot be made to these studies, as we altered fertilization levels of both macro- and micro-nutrients; whereas they only altered nitrogen levels. Also, the maize seedlings were only grown for 11-13 days, whereas the tobacco plants were grown for 40 days—though they altered fertilization regimes only 7 d before harvesting. In our study, gerbera plants were subjected to different fertilization regimes for the duration of the study (35 d).

The availability of potassium (K^+) also appears to play a role in JA biosynthesis. Armengaud et al. (2004) used full genome microarrays to assess transcriptional responses of *Arabidopsis* seedlings to changing external supply of potassium (K^+). The most prominent response was found for genes linked to jasmonic acid. K^+-starvation caused a strong increase in transcript levels for the JA biosynthetic enzymes lipoxygenase, allene oxide synthase, and allene oxide cyclase, and the expression of these genes were rapidly decreased in response to K^+ resupply. A large number of JA responsive genes were also up-regulated in response to K^+-starvation. Ashley et al. (2006) provided more evidence for K^+ deficiencies showing similarities to those

involved in stress responses to wounding and insect and pathogen attacks, in which JA and derivatives play a prominent role. By contrast, in nitrogen and phosphorus starved plants, no JA-responsive genes were identified by expression profiling of up to 8000 genes, although differential regulation of several defense-related transcripts was observed (Hammond et al., 2003; Wang et al., 2000, 2003; Wu et al., 2003). JA is known for its role in plant defense response against insect herbivores and (mainly necrotrophic) fungi, which are the most relevant enemies of K^+-starved plants (Kessler and Baldwin, 2002). Perhaps, the observed increase in JA serves to protect K^+-deficient plants against herbivore and fungal attack, while not entirely compensating for increased feeding and pathogen development. Potassium levels did not likely affect JA accumulation in this study, because K^+ levels were sufficient in both 0X and 1X plants. Nitrogen, and possibly phosphorus, levels were most likely the major influence on JA accumulation.

Some plants compensate for herbivore damage through rapid growth and development (Herms and Mattson, 1992; McNaughton, 1983; Retuerto et al., 2004). However, when nutrients are limiting, increased growth may not be an option. Hence, plants may allocate their resources towards defense in order to preserve plant tissue until environmental conditions improve. In gerbera, it appears that reducing fertilization favors secondary metabolism, and the subsequent increase in constitutive phenolic compounds that may deter or poison herbivores. In addition, jasmonic acid accumulation is also greater in 0X gerbera plants, which indicates that these plants are better prepared to initiate induced resistance mechanisms in response to herbivore

feeding. In nutrient stressed plants, JA accumulation may be greater and sustained because out-growing the damage is not plausible, and reducing herbivore damage takes priority.

The floriculture crop *Gerbera jamesonii* is an economically important crop with substantial fertilization recommendations. The recommended fertilization for potted gerbera is 100-150 $mg \cdot L^{-1}$ N for first two to three weeks, then 200-250 $mg \cdot L^{-1}$ N from a balanced fertilizer on a constant liquid fertilization basis (Kessler, 1999). Gerbera is also highly susceptible to a variety of insect pests, of which WFT are the most problematic. Hence, pesticide use on gerbera is prevalent. Based on the results of this study, reducing fertilization increases total phenolics (constitutive defense) and JA accumulation (induced defense). Optimizing fertilization may prove to be a useful tool in an integrated pest management (IPM) system. If fertilization is reduced to a level that increases host plant resistance, while producing marketable crops, then fertilizer run-off, pesticide usage, and associated chemical phytotoxicity (Spiers et al., 2006) could be reduced in greenhouse production systems. In order to ascertain the practicality of altering fertilization to increase host plant resistance in gerbera, the effects of fertilization on prominent insect pests, and the subsequent effects on plant quality, physiology, growth and development need to be determined.

Summary

This research focused on the effects of plant nutrition on jasmonic acid (JA) accumulation and total phenolic concentrations in *Gerbera jamesonii* 'Festival Salmon'. The phytohormone, JA, is known to regulate many plant responses, including inducible defenses against insect herbivores. Phenolics are constitutive secondary metabolites that have been shown to negatively affect insect feeding. Gerbera plants were grown in a growth chamber and subjected to either 0X fertilization (only supplied with initial fertilizer charge present in professional growing media) or 1X fertilization (recommended rate—200 mg L^{-1} N)]. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 or 200 mg L^{-1} N at each watering (as needed). Treatments consisted of ± mechanical wounding with a hemostat to one physiologically mature leaf and the subsequent harvest of that leaf at specified time intervals for JA quantification. Total phenolics were measured in physiologically mature and young leaves harvested 0 and 10 h after ± mechanical wounding. Plants receiving 0X fertility had reduced aboveground dry mass, were deficient in nitrogen and phosphorous, and had approximately a 10-fold higher concentration of total phenolics in physiologically mature leaf tissue compared to 1X plants. In both 0X and 1X plants, young leaves had greater concentrations of phenolics compared to physiologically mature leaves. There were no differences in total phenolics due to wounding. JA levels were significantly affected by plant nutrition. JA accumulation increased more rapidly and this accumulation was sustained longer in 0X plants. JA levels peaked 0.5 h after

wounding in 0X plants; and 1 h after wounding in 1X plants. In 0X plants, JA accumulation was significantly higher 0.5 h and 3 h after wounding, compared to 1X plants. There were no differences in levels of JA among non-wounded 0X and 1X plants. Hence, both constitutive (e.g. phenolics) and induced (e.g. JA) chemical defenses may be increased by reducing fertilization in gerbera.

CHAPTER IV

FERTILIZATION AFFECTS WESTERN FLOWER THRIPS FEEDING DAMAGE AND SUBSEQUENT ACCUMULATION OF JASMONIC ACID IN *Gerbera jamesonii*

Nutrition of host plants has been shown to have a direct effect on the productivity of numerous insect pests, including western flower thrips [(WFT) *Frankliniella occidentalis* (Pergande)] – a major pest on both ornamental and agricultural plants. Many insect herbivores are more prolific on plants treated with higher nitrogen fertility. The growth and reproduction of phytophagous insects can be limited by the nutritional content of their hosts, and generally increases as foliar nitrogen content increases (Mattson, 1980). In tomato (Stavisky et al., 2002) and chrysanthemum (Chau et al., 2005; Chau and Heinz, 2006; Davies et al., 2005; Schuch et al., 1998), WFT abundance decreased when fertilization was reduced. Likely, the effect of fertilization on essential elements of the WFT diet do not account for all of the observed results. However, the effects of fertilization on constitutive defenses (e.g. secondary metabolites) and induced defenses (e.g. JA accumulation) were not measured in any of these studies.

WFT are major insect pests of many herbaceous greenhouse crops because of their feeding damage and their role as vectors of tospoviruses. WFT are extremely opportunistic (Mound and Teulon, 1995), feed on a wide range of plant tissue types (Trichilo and Leigh, 1988) and can even be predacious (Gonzalez and Wilson, 1982;

Trichilo and Leigh, 1986). When WFT feed on developing tissues, the tissue becomes distorted because affected cells are unable to expand. WFT feeding damage on expanding leaf tissue has a 'silver' appearance because the cells are full of air (De Jager et al., 1995).

WFT are difficult to control because of their small size, ability to reproduce to high numbers, cryptic behavior, egg deposition inside plant tissue, and propensity to secrete themselves in tight spaces. Also, WFT appear to have a propensity for becoming resistant to insecticides. Many populations of WFT have shown resistance to a number of different insecticides, including insecticides with different chemical classes (Brøadsgaard, 1994; Espinosa et al., 2002; Immaraju et al., 1992; Zhao et al., 1995). This is partially due to the current reliance on insecticides for control of WFT. To avoid the development of insecticide resistance, producers need to reduce their reliance on insecticides and develop alternative management strategies. Ideally, an integrated pest management (IPM) system that combines host plant resistance, cultural practices, biological control, and chemical control should be employed to control WFT in greenhouse production. Precision fertilization is a cultural practice that may facilitate the control of WFT and reduce insecticide applications.

Previously, we determined the effects of fertilization on the level of total phenolics, which are constitutive secondary metabolites implicated in defense against phytophagous insects (Chapter III). Total phenolics were substantially increased (approximately 10-fold) in 0X (only received fertilizer charge present in growing mix) fertility gerberas compared to 1X (recommended fertility) fertility gerberas. In addition,

wound-induced jasmonic acid (JA) accumulated more rapidly and was sustained longer in 0X plants, compared to 1X plants. Jasmonates represent the best characterized class of signaling compounds mediating the elicitation of defense responses to wounding and herbivory, and they have been shown to be necessary for the induction of a wide array of defensive compounds. Prior to our research with gerbera, only two studies detailed the influence of nitrogen availability on JA accumulation. Interestingly, N deficiency was shown to affect JA accumulation in contrasting ways. In maize, wound- and elicitor-induced JA accumulation increased in low N plants, compared to high N plants (Schmelz et al., 2003b). In native tobacco (Nicotiana attenuata), wound- and elicitor-induced JA accumulation decreased in low N plants, compared to high N plants (Lou and Baldwin, 2004). Apparently, these species have developed different mechanisms to cope with N deficiency due to their evolutionary history.

In our earlier work, we mechanically wounded gerbera leaves with a hemostat and measured JA accumulation. The hemostat crushed leaf tissue –which is different than WFT feeding damage. Thrips are cell-content-feeding insects that penetrate single cells with a stylet to suck out the contents (Kindt et al., 2003). Recently, WFT feeding was shown to increase the accumulation of JA in *Arabidopsis* leaf tissue (De Vos et al., 2005). *Arabidopsis* plants were inoculated with 20 WFT larvae, and JA accumulation was determined up to 3 days after inoculation (the highest JA level was reportedly 72 h after inoculation). To my knowledge, there are no other documented reports of JA accumulation in response to WFT feeding.

Our previous research indicates that nutrient deficient gerberas have increased host plant resistance, but the actual effect on insect pests was not determined. Also, previously we used only two fertilization treatments; 1X (recommended rate) and 0X (only supplied with initial fertilizer charge present in commercial media). The 0X plants were nutrient deficient and would not be commercially acceptable. In this study, a moderately fertilized (0.3X) treatment was included to ascertain whether host plant resistance to WFT is affected by reducing fertilization by a more modest amount. The objectives of this study were to determine the effects of fertilization on WFT feeding, and the subsequent effects on JA accumulation.

Materials and Methods

Plant Cultural Conditions

Thirty rooted *Gerbera jamesonii* 'Festival Salmon' seedlings (Knox Nursery Inc., Winter Garden, FL) were transplanted into 6-inch standard pots (15.5 × 11.5 cm; 2050 cm^3) on 23 February 2007. Sunshine Mix #1 (Sun Gro Horticulture Inc., Pine Bluff, AK) was used as potting media. Each pot with one established seedling plug was a single replication. The plants were kept in a growth chamber at 24°C day/20°C night, 60% RH and grown under a 14 h photoperiod. Light intensity was approximately 300 μmol s^{-1} m^{-2}. The recommended fertilization for potted gerbera is 100-150 mg L^{-1} N for the first two to three weeks, then 200-250 mg L^{-1} N from a balanced fertilizer on a constant liquid fertilization basis (Kessler, 1999). In this experiment, three different

concentrations of fertilizer were supplied to gerberas that represented low fertilization [(0X), only supplied with initial fertilizer charge present in professional growing media – which is a 5-6-12 (elemental analysis: 5N-2.6P-10K) fertilizer incorporated at 2.7 lbs yd^{-3} (1.6 kg m^{-3}) of mix], moderate fertilization (0.3X), or recommended fertilization (1X). Plants were fertilized with a 200 ml fertilizer solution of Peters Professional Peat-lite special 15-16-17 (elemental analysis: 15N-7P-14.2K; Scotts-Sierra Horticultural Products Co., Marysville, OH) at 0 (de-ionized water), 60, or 200 $mg \cdot L^{-1}$ N, which are respectively, 0%, 30%, or 100% the recommended rate.

Western Flower Thrips Inoculation

Colonies of western flower thrips [(WFT) *Frankliniella occidentalis* (Pergande)] were established and maintained in the Texas A&M University Biological Control Laboratory at 26˚C, 65% RH with a 14 h photoperiod. One hundred WFT larvae were transferred, using a small soft-bristled paintbrush, to the adaxial surface of one physiologically mature leaf on day 41 (April 4, 2007) and allowed to feed for 3 days. Larvae were used for all experiments because, unlike adults, they are fairly immobile. The inoculated leaf was enclosed in a modified petri dish. The petri dish consisted of a 150 × 15 mm BD Falcon™ petri dish with 12 holes (approximately 15 × 15 mm) on each half of the petri dish for ventilation. Holes were formed by melting the plastic with a heated bolt. Square sections of thrips-proof nylon were glued on the inside of the petri dishes to cover the holes. Another hole (approximately 8 × 8 mm) was made on the side of the petri dish to allow for the leaf petiole to remain attached to the plant. Once placed

on the leaf and inoculated with ± WFT, a thin layer of petroleum jelly was applied to the lip of the bottom half of the petri dish to create a seal and deter WFT from escaping. After the leaf was enclosed within the petri dish, clear packaging tape and 2 large binder clips (50 × 25 mm) were used to seal the outside. Nontoxic, non-hardening, plastic clay was used to seal the hole around the leaf petiole. The lip of the pot, and/or additional pots supported the petri dish. Several preliminary trials were conducted to ensure that WFT could not escape before initiating the study.

Estimation of WFT Feeding Damage

Seventy-two h after inoculating (day 44) with ± WFT, the petioles of sample leaves were cut and pictures of each leaf were taken. Leaves were placed in a designated area, equidistant from the camera, and pictures (of adaxial and abaxial surfaces) were taken via remote shooting to avoid altering the fixed position of the camera. Leaf area and estimation of feeding damage was determined via Adobe® Photoshop™ and Scion® Image software using the methodology described by Chen and Williams (2006). The total leaf area of sample leaves and total damaged leaf area were calculated in units of actual pixels. The percent feeding damage (PFD) was calculated by dividing the total leaf area damaged by WFT feeding by the total leaf area of the sample leaf, then multiplying by 100.

Jasmonic Acid Quantification

After pictures were quickly taken, leaf samples (same leaves that were enclosed in petri dish) were frozen in liquid N_2 and stored at –80 ºC before analysis. JA was measured using isotope dilution based gas chromatography-mass spectrometry.

Extraction Procedure

Frozen tissue samples were ground to a fine powder in liquid nitrogen with mortar and pestle. A 'mastermix' was prepared that contained 5 mL of methanol and 50 ng of 1,3-[^{13}C]-JA internal standard (Creelman and Mullet, 1995) for each sample. The extraction 'mastermix' was divided into 15 mL Corning extraction tubes, warmed to 50°C, and kept warm in a beaker of water. Frozen tissue samples were weighed (0.8 - 2.5 grams) and placed in the extraction tubes, quickly mixed, and returned to the 50 °C water bath. Tubes were periodically shaken and returned to the water bath for 15 minutes. Then tubes were centrifuged for 5 minutes at 4000 rpm. The supernatant was carefully removed and dried. The extraction process was repeated until the supernatants and pellets were no longer green.

Sample Purification

Samples were initially purified by C_{18} solid phase extraction [C_{18} Bakerbond spe™ 3 mL extraction columns; packed with reversed phase Octadecylsilane (500 mg); J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ]. Columns were prepared by adding 3 mL of 80% methanol, and then preconditioned with 6 mL of 0.4% acetic acid. Samples were dissolved in 2 mL of 0.4% acetic acid and poured through column 3× (6

mL/sample). Column was washed 3× (6 mL) with same 0.4% acetic acid. Samples were eluted with 5 mL of 80% methanol and the eluate was dried.

Dried samples were re-suspended in 220 µL of filtered MeOH:HOAc (0.1 N) 35:65. Extracts were filtered through 0.2-mM syringe filters and 190 µL was applied to a C_{18} HPLC column (Alltech® alphaBond C_{18}, 300 x 3.9 mm, 125A, 10µ). Constituents were separated by HPLC on a linear gradient from 35% to 85% methanol in 0.1 N acetic acid, with a flow rate of 0.8 mL per minute. Fractions were collected based on elution times previously determined with authentic JA standards (23.2 to 24.8 min), and then dried.

Gas Chromatography-Mass Spectrometry

The dried fractions were re-dissolved in 100 µL of MeOH, transferred to labeled reacti-vials, and dried under N_2. Samples were then methylated by adding 50 µL of ethereal diazomethane, capping reacti-vials tightly, and vortexing. The capped vials were then allowed to rest in a shaded location under a fume hood for 20 minutes. After 20 minutes, the caps were removed and diazomethane was allowed to passively evaporate under the fume hood, until dry. The methylation procedure was then repeated. JA levels were determined by injecting samples into a Varian 3400 gas chromatograph equipped with a Saturn® 3 mass spectrometer. The sample was suspended in 15 µL of ethyl acetate and 5 µL was injected with a septum programmable injector (SPI) set at 220 °C. The column used was a DB-5 (30 m × 0.25 mm × 0.25 µm) with a temperature program of 60 °C to 250 °C at a rate of 15 °C min^{-1} (12.66 min), with helium as the carrier gas (constant flow rate 1 ml/min). The analytes were ionized by electron impact.

JA detections were confirmed by matching the retention time (R.T. ~ 10.02 min) and mass spectrum of JA standards to the unconfirmed analyte. Endogenous JA levels were determined by comparing ^{12}C JA peak areas (*m/z* 224) to the ^{13}C JA internal standard peak areas (*m/z* 226).

Macro- and Micro-Nutrient Analysis

Additional plants at 0X, 0.3X, and 1X fertility were used to determine nutrient content of leaf tissue. Physiologically mature leaves were harvested from each fertility treatment at the termination of the experiment (n=4). The mineral status of plants was then determined on a DM basis. The tissue samples were dried in a convection oven at 70ºC for 120 h. Dry leaves were ground in a Wiley mill. Nitrogen content was determined using the Kjeldahl procedure (Rund, 1984). The remaining samples were digested in wet acid (Jones et al., 1991) and macro and micro-element determinations were made on an inductively coupled plasma atomic emission spectrophotometer (3510 ICP) at a commercial lab (J.R. Peters/Scott's Testing Laboratory, Allentown, PA) using the procedures of Munter and Grande (1981).

Plant Growth

To characterize plant growth, the dry mass of aboveground plant tissue (vegetative and reproductive) was measured. Plants were cut at the soil line, dried in a convection oven at 70ºC for 120 h, and weighed.

Experimental Design

The experiment was arranged as a 3 fertility × 2 ± WFT factorial for JA accumulation and WFT feeding damage. Each pot with one established seedling was a single replicate. There were 5 replicates (n=5) arranged in a completely randomized design. The data was analyzed using ANOVA (SAS Institute Inc., 2000).

Results

Percent Feeding Damage Caused by WFT

WFT feeding was evident on all WFT-inoculated 0.3X- and 1X-treated leaf samples. WFT feeding damage included yellow-tan to dark brown spots of necrotic tissue on adaxial leaf surfaces. WFT feeding was negligible on abaxial leaf surfaces; hence, only the adaxial leaf surface (on which WFT larvae was transferred) was used to determine the percent feeding damage (PFD). The differences in the degree of WFT feeding among fertility treatments were visually apparent. The PFD increased as fertilization increased ($P \leq 0.0073$) (Fig. 4.1). There was no detectable WFT feeding damage on WFT-inoculated 0X leaves. The PFD on WFT-inoculated 0.3X leaves ranged from 0.4% to 1.3%, whereas, the PFD on WFT-inoculated 1X leaves ranged from 2.0 to 7.0% (Fig. 4.1).

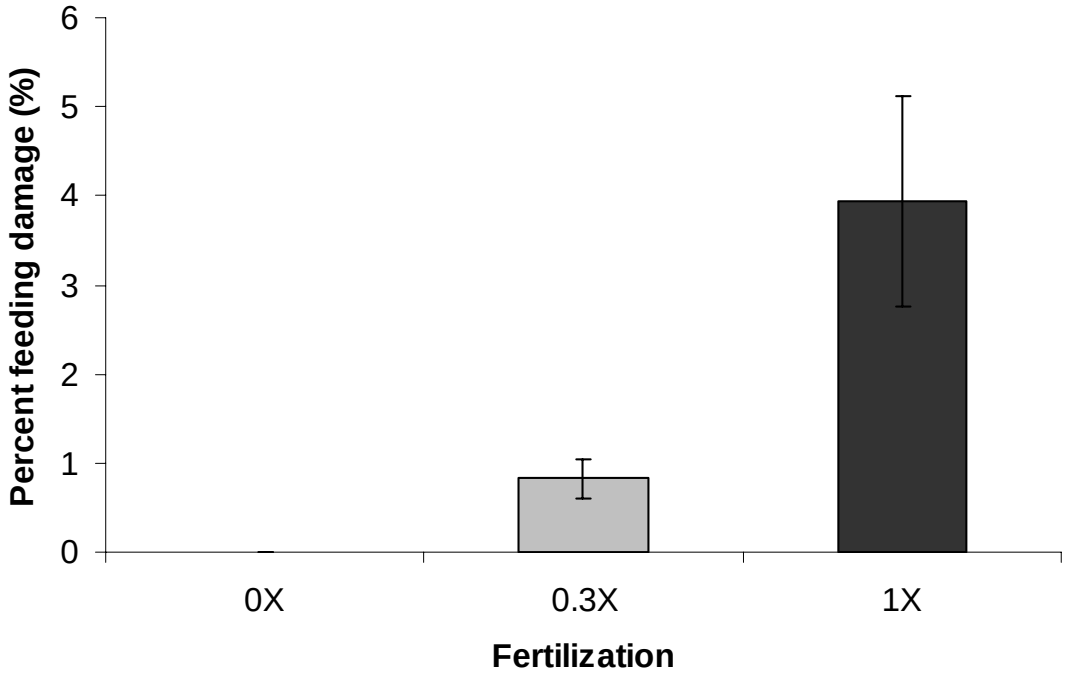

Fig. 4.1. The percent feeding damage (PFD) on physiologically mature leaves of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips, and treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) mg·L^{-1} N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$); ± SE, n = 5.

Jasmonic Acid Accumulation

The accumulation of jasmonic acid (JA) increased in response to WFT feeding ($P \leq 0.0035$) (Fig. 4.2). Fertilization ($P \leq 0.0405$) and the interaction of fertilization and WFT feeding ($P \leq 0.0395$) were also significant. JA levels were highest in 1X leaves inoculated with WFT at 94.6 ng g^{-1} fresh weight (FW), compared to 29.8 ng g^{-1} FW in 1X leaves without WFT. JA levels were also significantly higher in 0.3X leaves with WFT (62 ng g^{-1} FW), compared to 0.3X leaves without WFT (37 ng g^{-1} FW). Plants

receiving 0X fertility did not have any detectable WFT feeding damage; hence JA levels were similar for WFT-inoculated (47 ng g^{-1} FW) and non-inoculated (42 ng g^{-1} FW) 0X leaves. Constitutive levels of JA (non-inoculated leaves) tended to be higher as fertilization was reduced ($P \leq 0.0232$). Leaves of 0X plants contained significantly higher levels of JA compared to 1X plants; however, JA levels in 0.3X leaves were not significantly different than in 0X or 1X leaves.

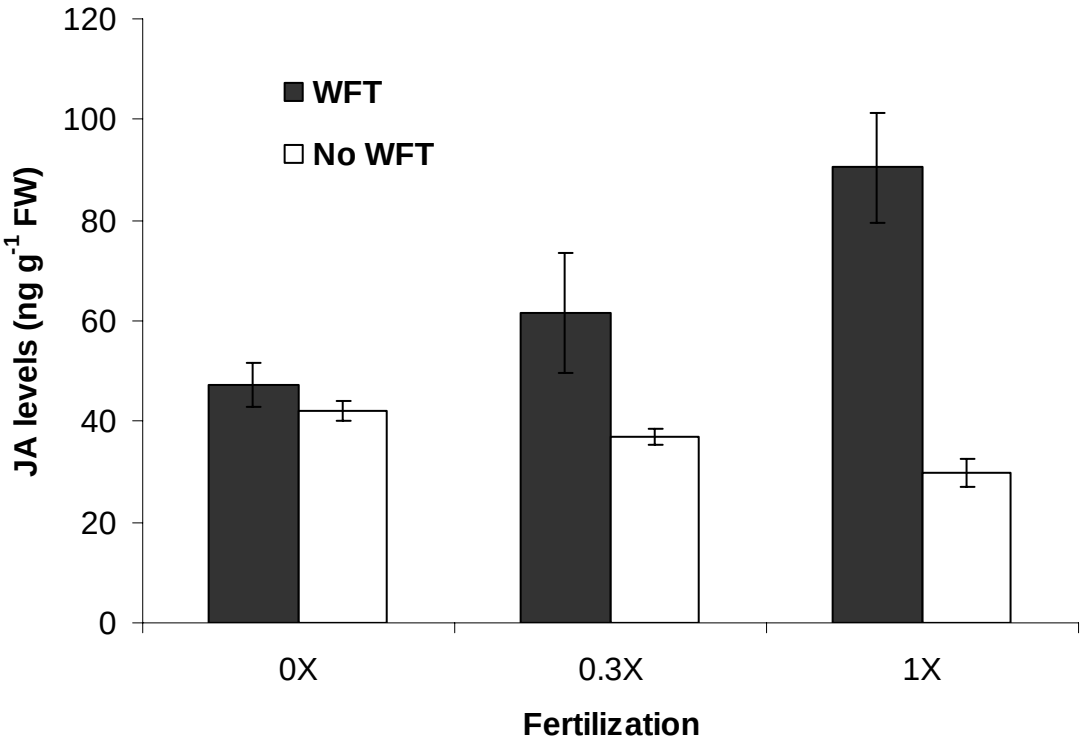

Fig. 4.2. The jasmonic acid concentration in physiologically mature leaves from *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) mg·L^{-1} N at each watering, as needed. Fertilization ($P \leq 0.0405$), WFT feeding ($P \leq 0.0035$), and the interaction fertilization × WFT ($P \leq 0.0395$) were significant; ± SE, n = 5.

The PFD of adaxial leaf surfaces was positively correlated with JA accumulation. In both 1X and 0.3X plants, as WFT feeding damage (i.e., PFD) increased, JA accumulation increased ($R^2 = 0.80$ for 1X; $R^2 = 0.80$ for 0.3X) (Fig. 4.3). Based on the slopes of the linear regressions, 0.3X plants accumulated 47.7 ng JA g^{-1} fresh weight (FW) each percent increase in WFT-induced PFD; whereas, 1X plants only accumulated 7.7 ng JA g^{-1} FW each percent increase in PFD. The 0X plants did not have any detectable feeding damage; hence, correlations could not be made.

Plant Growth and Nutrient Content

Plant growth (shoot DM) was substantially reduced in 0X plants, but was similar among 0.3X and 1X plants (Fig. 4.4). Fertilization was the only significant main effect ($P < 0.0001$); WFT feeding or the interaction among fertilization and WFT feeding did not affect plant DM.

With the exception of iron (Fe), fertilization had a significant effect on all macro- and micro-nutrients present in physiologically mature leaf tissue (Table 4.1). According to current recommendations for tissue nutrient content of high quality gerberas, 0X plants were deficient in N, P, and K (Dole and Wilkins, 1999). The 0.3X plants were deficient in N, but sufficient in all other nutrients.

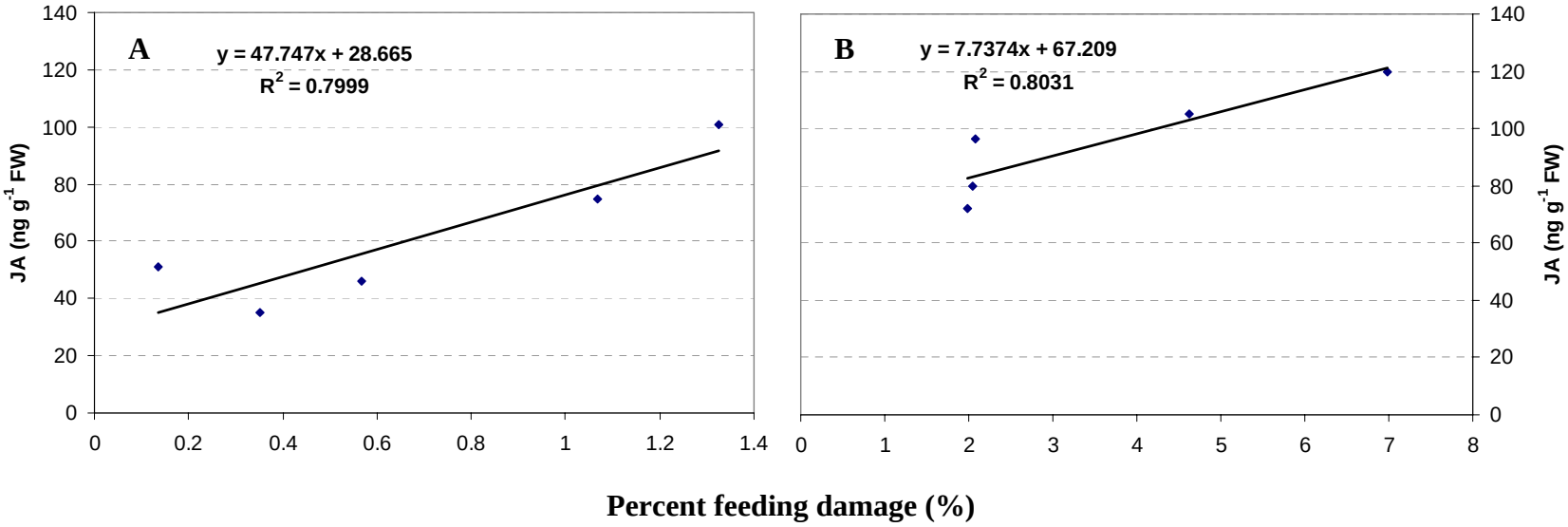

Fig. 4.3. **A**) The effect of WFT feeding damage on the accumulation of jasmonic acid (JA) in mature leaves of 0.3X fertility *Gerbera jamesonii* 'Festival Salmon'. **B**) The effect of WFT feeding damage on the accumulation of JA in mature leaves of 1X fertility *Gerbera jamesonii* 'Festival Salmon'. Percent feeding damage (PFD) is the amount of western flower thrips feeding damage/total leaf area. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) mg·L-1 N at each watering, as needed; n = 5. WFT did not cause detectable feeding damage on 0X fertility plants.

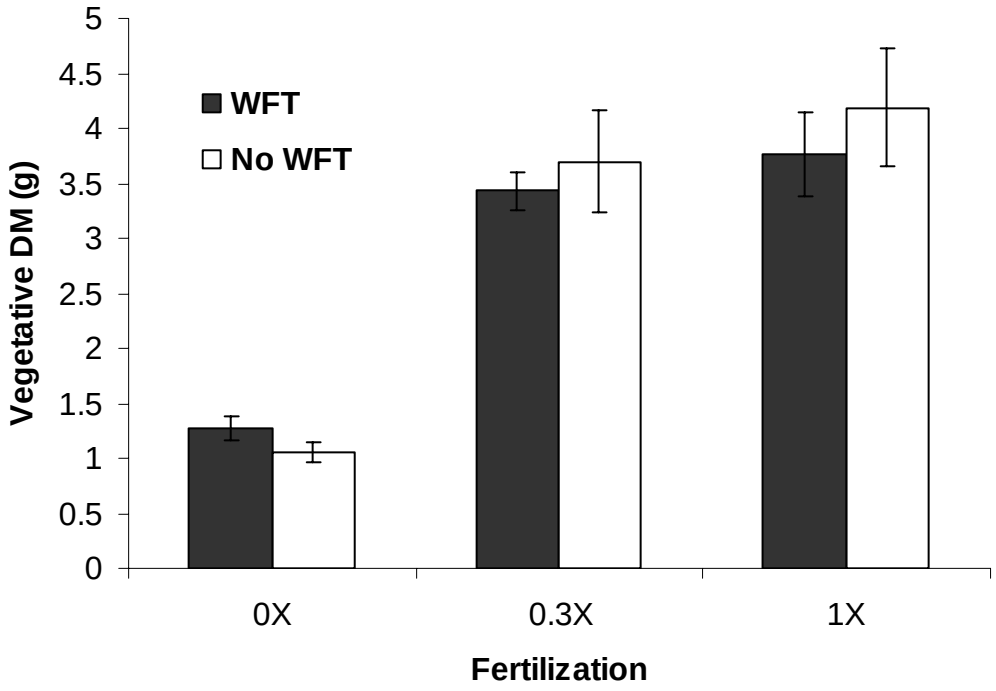

Fig. 4.4. The shoot dry mass (DM) of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), and treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, $n = 5$.

Table 4.1. Effect of fertilization application on leaf macro- and micro-nutrient content of *Gerbera jamesonii* 'Festival Salmon'. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed; n = 4. The recommended nutrient levels are general recommendations for tissue of high-quality greenhouse gerberas (Dole and Wilkins, 1999).

	Macro-nutrients						Micro-nutrients							
Fertilizer	N	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Zn	Mo	Na	Al
	%	%	%	%	%	%	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$
0X	0.8 ± 0.1	0.1 ± 0.0	2.4 ± 0.0	0.7 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	53.6 ± 4.9	50 ± 3.9	40.7 ± 2.9	4 ± 0.8	16.8 ± 0.7	0.7 ± 0.0	525 ± 55.9	33.1 ± 3.9
0.3X	2.2 ± 0.1	0.3 ± 0.0	3.1 ± 0.2	0.7 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	54.2 ± 3.0	39.3 ± 3.5	38.5 ± 1.2	1.9 ± 0.1	20.1 ± 1.6	1.1 ± 0.2	539 ± 36.5	19.4 ± 1.9
1X	3.6 ± 0.2	0.8 ± 0.1	4.1 ± 0.1	1.0 ± 0.1	0.5 ± 0.0	0.1 ± 0.0	64.1 ± 1.7	81 ± 2.6	70.9 ± 2.8	2.8 ± 0.1	26.6 ± 0.8	0.6 ± 0.0	962 ± 67.8	34.9 ± 4.8
Recom-mended	2.7 – 4.1	0.3 – 0.7	3.1 – 3.9	0.4 – 4.2	0.3 – 2.8	---	60 – 130	30 – 260	19 – 50	2 – 10	19 – 80	---	---	---
Fertilizer	< 0.001	< 0.0001	< 0.0001	0.0013	0.0009	< 0.0001	NS	< 0.0001	< 0.0001	0.0037	0.0005	0.0144	0.0004	0.0315

Discussion

This is the first study to determine that WFT feeding induces the accumulation of jasmonic acid (JA) in gerbera. In addition, nutrient availability had substantial effects on JA accumulation, and the amount of damage caused by WFT feeding. In our previous work, the concentration of total phenolics increased and wound-induced jasmonic acid (JA) accumulated more rapidly, and this increase in JA was sustained longer in low fertility (0X) plants, compared to recommended (1X) fertility plants (Chapter III). The goals of this current research were to determine the effects of fertility on WFT feeding, and the subsequent effects on the accumulation of JA. WFT feeding damage was substantially reduced on 0X plants, and reduced to a lesser extent on 0.3X plants. In addition, JA accumulation was proportionate to the amount of damage caused by WFT feeding.

JA levels increased in response to WFT feeding on 0.3X and 1X treatments. JA accumulates in response to physical damage to cell walls (often caused by herbivores) (Creelman and Mullet, 1997), and JA biosynthesis has been shown to be necessary for defense against both chewing and cell-content feeding (e.g. WFT) insects (Li et al., 2002). JA alters gene expression resulting in the accumulation of defense proteins such as proteinase inhibitors, which affect the digestive system of attacking herbivores (Farmer and Ryan, 1992; Reinbothe et al., 1994), or in the accumulation of low molecular weight compounds with antibiotic properties (phytoalexins) (Blechert et al., 1995). In addition to the role of JA in direct defense against insect herbivores, JA

accumulation may also function as an indirect defense. JA treated plants have been shown to increase volatile emissions that attract natural enemies, thus increasing the mortality rate of particular insect pests in gerbera (Gols et al., 1999), maize (Ozawa et al., 2004), native tobacco (Halitschke et al., 2000; Kessler and Baldwin, 2001), tomato (Thaler, 1999), and rice (Lou et al., 2005). Hence, if wound-induced JA accumulation is increased in lower fertility plants, plants may be better prepared to defend against insect pests either directly (e.g. increasing defensive compounds), or indirectly (by attracting natural enemies).

WFT feeding was reduced on gerberas that received fertilization rates representing 0% and 30% of the recommended fertilization rate. In fact, WFT were unable to survive on 0X gerbera plants (received only initial fertilizer charge present in commercial media), and no detectable WFT feeding was observed. The 0X plants were deficient in N, P, and K, and were noticeably stressed (chlorotic). The 0X gerberas contained high concentrations of phenolics (Chapter III), which can be toxic to phytophagous insects. WFT apparently found the 0X tissue unpalatable and abstained from feeding. When other protein sources are scarce, adult WFT may supplement their diet by feeding on thrips larvae (Van Rijn et al., 1995). It's possible that cannabolism occurred, but unlikely, because no live WFT (adults or larvae) were observed after 72 h on the 0X plants. The observed results are likely due to a combination of chemical defenses and the reduced nutritional value present in leaf tissue of 0X plants. The 0.3X gerberas (received 30% of recommended rate) had similar biomass compared to 1X plants, and these plants did not appear to be stressed. The 1X plants had the highest

percentage of WFT feeding damage, and the damage was positively correlated to JA accumulation. There was significantly less feeding damage on 0.3X plants—which also had greater JA accumulation in response to WFT feeding, and the increase was positively correlated to WFT feeding damage (PFD).

Based on the rate of JA accumulation, the 0.3X gerberas appear to be more sensitive to WFT feeding than 1X plants. The 0.3X plants accumulated substantially more JA relative to the amount of WFT feeding damage (47.7 ng g^{-1} FW for 1 percent increase in PFD), compared to 1X plants (7.7 ng g^{-1} FW for 1 percent increase in PFD). In addition, JA levels in non-wounded leaves were significantly higher in 0X plants, when compared to 1X plants, though constitutive JA levels in 0.3X plants were not significantly different compared to 1X or 0X plants. Though statistically significant, the minute differences in constitutive JA levels may be misleading, as these results were not established in our other studies (see Chapter III and Chapter V).

The PFD ranged from 0.4 to 1.3% on 0.3X plants, and 2.0 to 7.0% on 1X plants. Fertilization affected the amount of feeding, and likely the duration or time of WFT feeding. WFT fed less on 0.3X plants compared to 1X plants, but none the less, PFD correlated well with JA accumulation within both fertility treatments (R^2 = 0.80 for 0.3X; R^2 = 0.80 for 1X) (Fig. 4.3). Of course, WFT-induced JA accumulation is not likely infinitely linear, and JA accumulation would subside at some point. However, when WFT feeding damage is relatively low, 0.3X gerberas seem to accumulate more JA compared to 1X gerberas.

The observed reduction in WFT feeding damage on lower fertility gerberas is likely due, in part, to an increase in secondary metabolites involved in defense against insect herbivores. In a previous study, the total phenolic concentration was approximately ten-fold greater in 0X gerberas, compared to 1X plants. Phenolics are the dominant allelochemicals in many plants and are known to decrease insect growth, development and survival (English-Loeb et al., 1997; Isman and Duffey, 1982; Stamp 1990; Wilkens et al., 1996). Inbar et al. (2001) reported a negative association between plant growth and chemical defense, which supports the growth-differentiation balance hypothesis (GDBH), described by Herms and Mattson (1992). The GDBH is premised upon a physiological trade-off between growth and differentiation processes. Secondary metabolism is physiologically constrained in dividing and enlarging cells, and requires photosynthates that could be used for growth processes. Plants have limited resources to support their physiological processes, and all requirements cannot be met simultaneously. Hence, trade-offs occur among growth, storage, reproduction and defense (Lambers and Poorter, 1992). Inbar et al. (2001) observed a positive association between insect performance and plant growth. Plants subjected to nutrient stress had higher levels of total phenolics and peroxidase, which negatively affected the prolificacy of insect pests (whitefly, leafminer, and corn earworm).

Based on the results of this study and previous research on the effects of fertilization on endogenous chemical defenses (Inbar et al., 2001; Schmelz et al., 2003b; Stout et al., 1998), the demand for defense signaled by insect feeding may override physiological constraints. It seems that the strategy for some plant species under

nutrient stress is to increase constitutive defenses, while maintaining, or possibly increasing inducible defenses instead of growth. Schmelz et al. (2003b) found that low N maize accumulated more JA than high N maize, and the increased JA combined with the increased sensitivity to ethylene resulted in increased volatile emission (indirect defense). Low N tomato plants were shown to have greater chemical defenses, compared to high N plants (Inbar et al., 2001; Stout et al., 1998). Similar to our previous findings, phenolics were substantially greater in low N plants. More importantly, JA-inducible N-containing chemical defenses were not compromised. The constitutive levels of peroxidase, polyphenol oxidase, and proteinase inhibitors were greater in low N plants than in high N plants (Inbar et al., 2001; Stout et al., 1998). Endogenous JA was not measured in these studies with tomato, but greater constitutive amounts of JA in low N plants, as demonstrated in 0X gerberas, may account for the increased constitutive amounts of these JA-inducible proteins. There were no substantial differences in the induced activity of polyphenol oxidase and proteinase inhibitors among low-N and high-N plants (Stout et al., 1998). These results provide evidence for an active damage-induced increase in allocation towards defense that overrides the physiological constraints imposed by low nutrient availability.

Our results support previous findings that nutrient availability affects the productivity of WFT (Chau et al., 2005; Chau and Heinz, 2006; Davies et al., 2005; Schuch et al., 1998; Stavisky et al., 2002). Our results indicate that reducing fertility increases plant compounds implicated in defense against insect herbivores, and this increase in host plant resistance is realized in reduced WFT feeding. The 0.3X gerberas

did not appear significantly stressed, yet the PFD was significantly reduced. The reduction in WFT feeding may have been due, in part, to greater concentrations of constitutive and induced chemical defenses. Low fertility plants have greater concentrations of phenolics, which are known to deter phytophagous insects. In addition, 0.3X gerberas accumulated more JA relative to WFT feeding damage. Precision fertilization reduction may be a viable tool in commercial gerbera production that enhances host plant resistance to WFT, yet produces a marketable crop. In order to test the feasibility of this hypothesis, future research is needed to determine the effects of fertility on WFT abundance in potted gerbera production, and the subsequent effects on plant physiology and quality.

Summary

Our previous research indicates that endogenous chemical defenses are increased when fertilization is reduced, but the actual effect on insect pests was not tested. The objectives of this study were to determine the effects of fertilization on WFT feeding and the subsequent effects on the accumulation of jasmonic acid (JA)—which is a phytohormone necessary for the induction of a wide array of defensive compounds. Thirty gerbera (*Gerbera jamesonii* 'Festival Salmon') seedlings were fertilized with concentrations that consisted of 0%, 30%, or 100% (200 mg L^{-1} N) the recommended fertilization rate for gerbera. On day 41, ± 100 western flower thrips [(WFT) *Frankliniella occidentalis* (Pergande)] larvae were transferred to the adaxial surface of

one physiologically mature leaf and allowed to feed for 72 h (n = 5). The inoculated leaf was enclosed in a modified petri dish that allowed for air flow, while preventing WFT from escaping the sample leaf. The percent feeding damage (PFD) increased as fertilization increased ($P \leq 0.0073$). There was no detectable WFT feeding damage on WFT-inoculated 0X fertility (only supplied with initial fertilizer charge in commercial media) plants. The PFD on 0.3X fertilized (30% recommended rate) gerberas inoculated with WFT ranged from 0.4 to 1.3%, whereas, the PFD on WFT-inoculated 1X fertility (100% recommended rate) leaves ranged from 2.0 to 7.0%. The accumulation of JA was significantly higher in response to WFT feeding, with JA levels greatest in 1X leaves inoculated with WFT. Constitutive levels of JA (non-inoculated leaves) tended to be higher as fertilization was reduced ($P \leq 0.0232$). The PFD of adaxial leaf surfaces was positively correlated with JA accumulation. In both 0.3X and 1X plants, as WFT feeding damage increased, JA accumulation increased ($R^2 = 0.80$ for 0.3X; $R^2 = 0.80$ for 1X). Based on the slopes of the linear regressions, 0.3X plants accumulated 47.7 ng JA g^{-1} fresh weight (FW) each percent increase in WFT-induced PFD; whereas, 1X plants only accumulated 7.7 ng JA g^{-1} FW each percent increase in PFD. Lower fertility gerberas were less susceptible to WFT feeding due, in part, to increased levels of chemical defenses.

CHAPTER V

THE INFLUENCE OF FERTILIZATION ON WESTERN FLOWER THRIPS, PLANT GROWTH, DEVELOPMENT, AND QUALITY OF *Gerbera jamesonii*

Gerbera jamesonii Bolus ex Hooker f. is an economically important floriculture crop that is sold as cut flowers, bedding plants, or as potted flowering plants. In the U.S., floriculture crop sales were $5.4 billion in 2005 (Floriculture and Nursery Crops Yearbook, 2006). Gerbera daisies sold as cut flowers represented almost 1% of the U.S. sales for cut flowers, with over $32 million in sales for 2005. Unfortunately, gerberas make excellent hosts for insect pests, especially western flower thrips [(WFT) *Frankliniella occidentalis* Pergande]. Gerberas have even been suggested for use as a 'trap crop' for managing WFT (Blumthal et al., 2005).

WFT are difficult to control because of their small size, ability to reproduce in high numbers, cryptic behavior, egg deposition inside plant tissue, and tendency to secrete themselves in tight spaces. Also, WFT appear to have a propensity for becoming resistant to insecticides. Many populations of WFT have shown resistance to a number of different insecticides, including insecticides with different chemical classes (Brøadsgaard, 1994; Espinosa et al., 2002; Immaraju et al., 1992; Zhao et al., 1995). This is partially due to the current reliance on insecticides for control of WFT. To avoid the development of insecticide resistance, producers should reduce their dependence on insecticides and develop alternative management strategies. Ideally, an integrated pest

management system that combines host plant resistance, cultural practices, biological control, and chemical control should be employed to control WFT in greenhouse production (Robb and Parrella, 1995). Modifying fertilization is a cultural practice that may facilitate in the control of WFT and reduce insecticide applications.

Many insect herbivores are more prolific on plants treated with higher nitrogen (N) fertilization (Inbar et al., 2001). High fertility has been shown to increase the productivity of WFT. Fertilizer rates representing 0%, 10%, 20% and 100% the recommended rate of N applied to chrysanthemum resulted in an increase in WFT populations as fertilization rates increased (Davies et al., 2005). In tomato, the actual N content in the flowers correlated with WFT abundance (Brodbeck et al., 2001). In separate studies, applying fertilizers with higher than recommended rates of N resulted in an increase in WFT populations in chrysanthemum (Schuch et al., 1998) and tomato (Stavisky et al., 2002). In contrast, Chau and Heinz (2006) reported significantly lower levels of WFTs on chrysanthemums fertilized at $\leq 50\%$ or $\geq 150\%$ the recommended level. Chen et al. (2004) reported that supplying a luxurious amount of N to impatiens had no affect on WFT populations when compared to impatiens receiving an optimum level of N. However, a similar increase in phosphorus resulted in more adult WFT four weeks after inoculation – indicating that excess phosphorus accumulation, but not N, may support more rapid growth of WFT populations in impatiens. Whether supra-optimal levels of fertilization affect WFT populations may be dependent on the plant-fertilizer combination. In general, lowering fertilization below recommended levels for chrysanthemum and tomato reduces the prolificacy of WFT.

The reduction in WFT abundance in response to lower fertility regimes has been attributed to the reduced availability of essential nutrients for WFT, and possibly secondary metabolites. However, the effects of fertilization on constitutive defenses (e.g. secondary metabolites) and induced defenses (e.g. jasmonic acid accumulation) was not measured in any of these studies. In a recent study, it was found that reducing fertilization increased constitutive total phenolics in *Gerbera jamesonii* (Chapter III). Phenolics are secondary metabolites that have been shown to negatively affect insect herbivory (Inbar et al., 2001). Secondary metabolism, which involves specialized, often complex and species-specific biosynthetic pathways, is thought to provide compounds which are accumulated and stored, so that when attacked, the plant is already provided with the means to deter, or kill herbivores. Toxic compounds, such as phenolics, can poison generalist herbivores and force specialist herbivores to invest resources in detoxification mechanisms that in turn incur growth and development costs (Kessler and Baldwin, 2002). Plant parts that are of high fitness value or that are under a high risk of attack may be best protected by constitutive defenses, whereas others may be better defended by induced responses (Wittstock and Gershenzon, 2002).

Low fertility gerberas were also shown to accumulate jasmonic acid more rapidly, and sustain this increase longer than recommended fertility plants (Chapter III). WFT feeding induced the accumulation of JA in gerbera, and the increase was proportionate to WFT feeding damage (Chapter IV). In addition, plants receiving 30% of the recommended fertility accumulated more JA relative to the amount of WFT feeding damage than recommended fertility plants. The phytohormone, jasmonic acid

(JA), accumulates in response to wounding (often caused by insect feeding), and plays a major role in defense against herbivores. Tomato mutants deficient in wound- and systemin-induced JA accumulation are more susceptible to attack by the chewing insect *M. sexta* larvae (Howe et al., 1996; Lightner et al., 1993). Very similar results were found using these mutant plants and the cell content-feeding, two-spotted spider mite (*Tetranychus urticae* Koch) (Li et al., 2002). Li et al. (2002) also showed that transgenic tomato plants that constitutively activate the octadecanoid pathway (pathway for JA biosynthesis) were highly resistant to attack by spider mites and western flower thrips (*Frankliniella occidentalis*).

There is wide interest in implementing best management practices (BMP) in greenhouse crop production. The emphasis is on reducing inputs such as fertilization (and subsequent fertilizer run-off), water (due to the growing water shortages), and pesticides. The U.S Environmental Protection Agency (EPA), in enforcing the 1972 Federal Clean Water Act, requires all States to implement a total maximum daily load program for all watersheds (Lea-Cox, 2001). Knowing the influence of fertilizer application on host plant resistance, plant quality, and pest populations may lead to optimized fertilization that maintains high crop quality, while minimizing insect damage and the need for intensive pesticide use, with associated chemical phytotoxicity (Spiers et al., 2006). Precision chemical usage can also reduce fertilizer and pesticide run-off, and contamination of surface and ground water (Yeager et al., 1997).

Our previous research indicates that lower fertility gerberas have increased chemical defenses, and are less susceptible to WFT feeding. The objectives of this study

were to determine the effects of fertilization on WFT abundance, and to characterize the effects of fertilization and WFT feeding on various plant growth and physiological characteristics. The effects of fertilization and WFT feeding on gerbera plant quality was also determined to assess the viability of altering fertilization in order to increase host plant resistance in gerbera.

Materials and Methods

Plant Cultural Conditions

Thirty rooted *Gerbera jamesonii* 'Festival Salmon' seedlings (Knox Nursery Inc., Winter Garden, FL) were transplanted into 6-inch standard pots (15.5 × 11.5 cm; 2050 cm^3) on 30 June, 2006. Sunshine Mix #1 (Sun Gro Horticulture Inc., Pine Bluff, AK) was used as potting media. Each pot was enclosed in a large acetate cylindrical cage (36 cm diameter, 61 cm high) constructed with Lexan® film (GE Polymershapes, Hunterville, NC) with a sealed top and bottom. Two openings (20 cm in diameter) were covered with nylon organdy cloth for ventilation. Clear PVC tubing (0.95 cm diameter, 26 cm long; VWR International, Suwanee, GA) was attached to each cage for irrigation and fertigation. The caged plants were kept in growth chambers at 24°C day/20°C night, 60% RH and grown under a photoperiod of 14L: 10D. Light intensity was approximately 300 μmol s^{-1} m^{-2}. The recommended fertilization for potted gerbera is 100-150 mg·L^{-1} N for first two to three weeks, then 200-250 mg·L^{-1} N from a balanced fertilizer on a constant liquid fertilization basis (Kessler, 1999). In this experiment, three

different concentrations of fertilizer were supplied to gerberas that represented low fertilization [(0X), only supplied with initial fertilizer charge present in professional growing media – which is a 5-6-12 (elemental analysis: 5N-2.6P-10K) fertilizer incorporated at 2.7 lbs yd^{-3} (1.6 kg m^{-3}) of mix], moderate fertilization (0.3X), or recommended fertilization (1X). Plants were fertilized with a 200 ml solution of Peters Professional Peat-lite special 15-16-17 (elemental analysis: 15N-7P-14.2K; Scotts-Sierra Horticultural Products Co., Marysville, OH) at 0 (de-ionized water), 60, or 200 $mg \cdot L^{-1}$ N, which are respectively, 0%, 30%, or 100% the recommended rate.

To protect against powdery mildew, plants were sprayed with the fungicide, propiconazole (Banner Maxx®, Syngenta Crop Protection, Inc., Greensboro, North Carolina) at the rate of 0.469 mL L^{-1} seven days after transplanting.

WFT Culture, Inoculation and Determination

Colonies of western flower thrips [(WFT) *Frankliniella occidentalis* (Pergande)] were established and maintained in the Texas A&M University Biological Control Laboratory at 26˚C, 65% RH with a 14 h photoperiod. Plants were caged and half (15 plants) were inoculated with WFT 19 days after transplanting (18 July, 2006). Five adult female WFT in a 1.5 ml micro-centrifuge tube (Scientific Inc., Ocala, Florida) were released near the base of the plant in each pot. The experiment was terminated after 53 d (31 August, 2006). At harvest, plants were cut at the soil line and placed in sealed plastic containers (25 cm long × 25 cm wide × 10 cm deep). Containers were shaken and individual flowers were dissected to dislodge any WFT. All WFT (from

vegetative and floral growth) were removed using an aspirator, stored in 70% alcohol and counted.

Plant Growth Measurements

Vegetative dry mass (DM), leaf area, specific leaf area (SLA, $cm^2\ g^{-1}$ DM of leaves), and total aboveground plant DM were measured at the termination of the experiment (day 53). Both vegetative and reproductive plant parts were included in the total aboveground DM measurements. Plants were cut at the soil line, dried at 70 ˚C for 96 h, and weighed to determine DM.

Flower Development

Flower number, flower DM, and reproductive DM (flowers, flower buds, and peduncles) were determined at the termination of the experiment to assess the effects of fertilization and WFT feeding on flower production. The rate of flower development was determined by recording the number of days from transplanting to pollen shed of the most mature flower. The DM of peduncles per individual open flower was determined to demonstrate the phenotypic plasticity of plants under different fertilization regimes.

Plant Quality Characteristics

Plant quality was determined by rating each plant (1 to 5, with 5 = optimal). The quality rating was based on marketability, and thus overall aesthetic quality was determined. Gerberas were rated for quality on day 54, based on the following scale: 1 =

very poor, unsaleable (extremely chlorotic and/or extensive WFT feeding damage); 2 = poor, unsaleable (chlorotic and/or WFT damage, few or no flowers); 3 = average, saleable (≤ 30% of media showing, lighter green and/or moderate WFT damage); 4 = good, saleable (foliage covering media, green, flowers, may have slight WFT damage); 5 = excellent, saleable (foliage covering media, dark green, no WFT damage to vegetation or flowers). Statistical significance for the quality rating was determined with a two-way ANOVA design for ranked data—the Scheirer-Ray-Hare extension of the Kruskal-Wallis Test (Sokal and Rohlf, 1995).

Jasmonic Acid Quantification

At the termination of the experiment (d 53), physiologically mature leaves were harvested for JA analysis. Tissue samples for JA were frozen in liquid N_2 and stored at –80ºC before analysis. JA was measured using isotope dilution based gas chromatography-mass spectrometry.

Extraction Procedure

Frozen tissue samples were ground to a fine powder in liquid nitrogen with mortar and pestle. A 'mastermix' was prepared that contained 5 mL of methanol and 50 ng of 1,3-[^{13}C]-JA internal standard (Creelman and Mullet, 1995) for each sample. The extraction 'mastermix' was divided into 15 mL Corning extraction tubes, warmed to 50°C, and kept warm in a beaker of water. Frozen tissue samples were weighed (1 – 2.5 grams) and placed in the extraction tubes, quickly mixed, and returned to the 50 °C water bath. Tubes were periodically shaken and returned to the water bath for 15

minutes. Then tubes were centrifuged for 5 minutes at 4000 rpm. The supernatant was carefully removed and dried. The extraction process was repeated until the supernatants and pellets were no longer green.

Sample Purification

Samples were initially purified by C_{18} solid phase extraction [C_{18} Bakerbond spe™ 3 mL extraction columns; packed with reversed phase Octadecylsilane (500 mg); J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ]. Columns were prepared by adding 3 mL of 80% methanol, and then preconditioned with 6 mL of 0.4% acetic acid. Samples were dissolved in 2 mL of 0.4% acetic acid and poured through column 3× (6 mL/sample). Column was washed 3× (6 mL) with same 0.4% acetic acid. Samples were eluted with 5 mL of 80% methanol and the eluate was dried.

Dried samples were re-suspended in 220 µL of filtered MeOH:HOAc (0.1 N) 35:65. Extracts were filtered through 0.2-mM syringe filters and 190 µL was applied to a C_{18} HPLC column (Alltech® alphaBond C_{18}, 300 x 3.9 mm, 125A, 10µ). Constituents were separated by HPLC on a linear gradient from 35% to 85% methanol in 0.1 N acetic acid, at 0.8 mL per minute. Fractions were collected based on elution times previously determined with authentic JA standards (23.2 to 24.8 min), and then dried.

Gas Chromatography-Mass Spectrometry

The dried fractions were re-dissolved in 100 µL of MeOH, transferred to labeled reacti-vials, and dried under N_2. Samples were then methylated by adding 50 µL of ethereal diazomethane, capping reacti-vials tightly, and vortexing. The capped vials were then allowed to rest in a shaded location under a fume hood for 20 minutes. After

20 minutes, the caps were removed and diazomethane was allowed to passively evaporate under the fume hood, until dry. The methylation procedure was then repeated. JA levels were determined by injecting samples into a Varian 3400 gas chromatograph equipped with a Saturn® 3 mass spectrometer. The sample was suspended in 15 µL of ethyl acetate and 5 µL was injected with a septum programmable injector (SPI) set at 220 °C. The column used was a DB-5 (30 m × 0.25 mm × 0.25 µm) with a temperature program of 60 °C to 250 °C at a rate of 15 °C min^{-1} (12.66 min), with helium as the carrier gas (constant flow rate 1 ml/min). The analytes were ionized by electron impact. JA detections were confirmed by matching the retention time (R.T. ~ 10.02 min) and mass spectrum of JA standards to the unconfirmed analyte. Endogenous JA levels were determined by comparing ^{12}C JA peak areas (*m/z* 224) to the ^{13}C JA internal standard peak areas (*m/z* 226).

Total Phenolics Quantification

Physiologically mature leaf tissue was harvested from each plant at the termination of the experiment (day 53) for total phenolic determination. Total phenolic content of leaf tissue was evaluated based on a method adapted from Swain and Hillis (1959), which describes the Folin-Ciocalteau reagent assay utilizing chlorogenic acid for a standard curve. In brief, fresh leaf tissue was weighed and ground with mortar and pestle in 80% methanol (6 mL). Extracts were centrifuged at 14,000 rpm for 15 minutes and placed in freezer (-80ºC) for two days. Reaction mixture consisted of mixing 30 µL of the extract with 90 µL of Na_2CO_3 and 150 µL of Folin-Ciocalteau reagent in a 96-well

microplate. After 30 min the absorbance was measured at 725 nm using a KC-4 spectrophotometer (Biotek® Instruments, Inc. Winooski, VT). Results were expressed as milligrams of chlorogenic acid equivalents per gram of fresh weight tissue.

Chlorophyll Determination

Leaf chlorophyll was determined with a SPAD-502 portable chlorophyll meter (Minolta Camera Co. LTD, Japan). The SPAD-502 meter readings were correlated with a chlorophyll content prediction equation: $y = 1.4929x - 12.979$, where y = chlorophyll content ($\mu g\ cm^{-2}$), x = meter reading ($R^2 = 0.9683$). This equation was obtained by running a linear regression analysis between the SPAD-502 readings obtained in a separate fertility study with physiologically mature leaves from five pots per fertility treatment (six treatments); and the total chlorophyll concentration of the same leaves (J.D. Spiers, unpublished data). Leaf chlorophyll was extracted with N,N-dimethylformamide (DMF) and the total concentration was determined by the optical density of filtered aqueous supernatant, which was measured at 647 nm and 664 nm with a spectrophotometer (Moran, 1982). Each leaf was a single replication and one physiologically mature leaf was randomly selected from each pot and measured at the end of the experiment with the SPAD-502 meter (n = 5).

Net Photosynthesis and Stomatal Conductance

Net photosynthesis (*Pn*) and stomatal conductance (g_s) measurements of individual leaves were taken at the end of the experiment (day 53) between 11:00 and

15:30 using a LI-6400 Portable Photosynthesis System (LI-COR Inc., Lincoln, NE). A fixed substrate level of 360 μL L^{-1} CO_2 was provided with a 12-gram cartridge, and the light source was an LED 6400 R/B at 600 μmol m^{-2} s^{-1}. Each leaf was a single replication, and there were 5 replications per treatment (n = 5).

Carbon/Nitrogen Ratio

Leaf tissue from physiologically mature leaves was harvested from each treatment at the end of the experiment for carbon/nitrogen analysis (n = 5). Total carbon and nitrogen were determined using a NA1500 C/H/N Analyzer (Carlo Erba Strumentazione, Milan, Italy) via Micro-Dumas combustion analysis at the Stable Isotope/Soil Biology Laboratory (SISBL, Institute of Ecology, University of Georgia, Athens, GA).

Experimental Design and Statistical Analysis

The experiment was arranged in a 3 fertility × 2 ± WFT factorial. Each pot with one established-rooted seedling is a single replicate. There were five replications (n = 5) arranged in a completely randomized design. Data were compared using analysis of variance (ANOVA) (SAS Institute Inc., 2000), with fertilization, WFT, and fertilization × WFT as main effects. When appropriate, mean separations were performed using Fisher's protected least significant difference test to determine treatment differences ($P \leq 0.05$). WFT infested and uninfested (control) plants were grown in two separate chambers to avoid cross-contamination. The experiment was repeated and chambers

switched between WFT treatments to avoid possible chamber effects. There were none. As the experiment was repeated twice, only results from the second experiment are presented as they mirrored the results from the first experiment.

Results

WFT Abundance

The total number of WFT increased as fertility levels increased from 1.1 on 0X fertility plants to 77.7 on 1X fertility plants ($P < 0.0001$; Fig 5.1). The 0.3X fertilized plants (received 30% of the recommended fertilizer rate) averaged 52.1 WFT, which is a 33% reduction in total WFT population compared to 1X plants. Many WFT were unable to survive on the 0X plants – three out of five 0X plants inoculated with WFT had no surviving WFT at the termination of the experiment. Dead WFT were observed on adaxial leaf surfaces of the 0X plants.

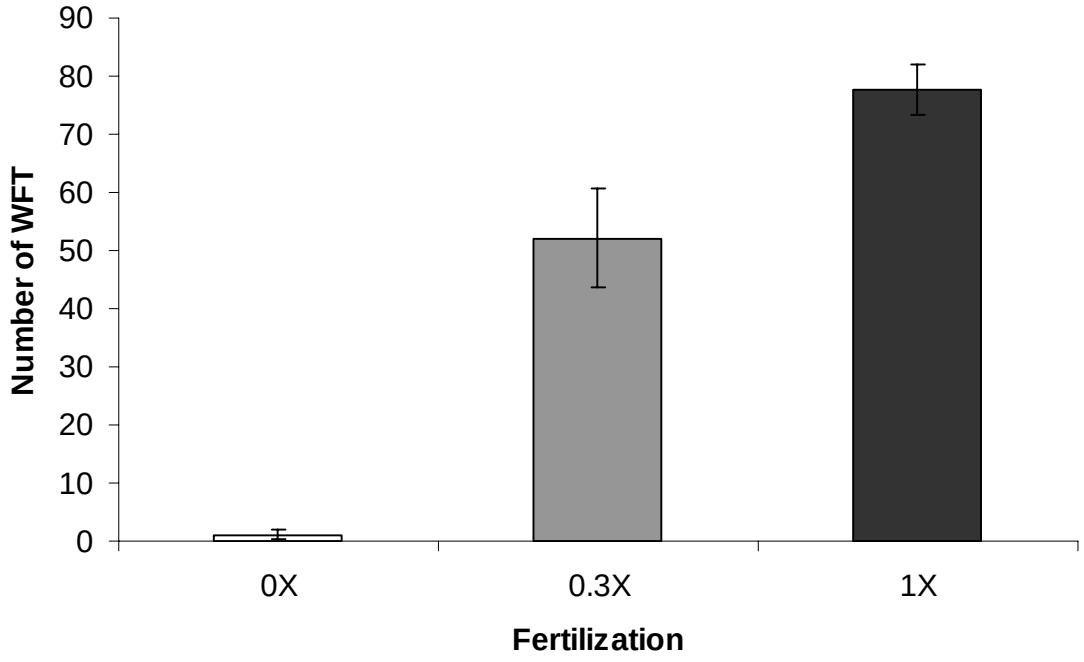

Fig. 5.1. The total number of western flower thrips [(WFT) *Frankliniella occidentalis* (Pergande)] on *Gerbera jamesonii* 'Festival Salmon' treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effect of fertilization was highly significant ($P < 0.0001$); ± SE, n = 5.

Plant Growth

Vegetative DM and total aboveground (vegetative and reproductive) DM were reduced in plants subjected to 0X and 0.3X fertilization rates ($P < 0.0001$) (Figs. 5.2; 5.3). Neither WFT feeding, nor the interaction among fertilization and WFT feeding affected plant biomass. Leaf area and specific leaf area (SLA) were also reduced in response to 0X and 0.3X fertilization rates (Figs. 5.4; 5.5). Fertilization ($P < 0.0001$) and the interaction among WFT feeding and fertilization ($P \leq 0.0001$) affected SLA, but WFT feeding was not a significant main effect. WFT feeding decreased the SLA of

0.3X and 1X plants, indicating that these leaves were thicker in response to the stress of WFT feeding. With such a low survival rate, WFT feeding did not significantly affect SLA in 0X plants.

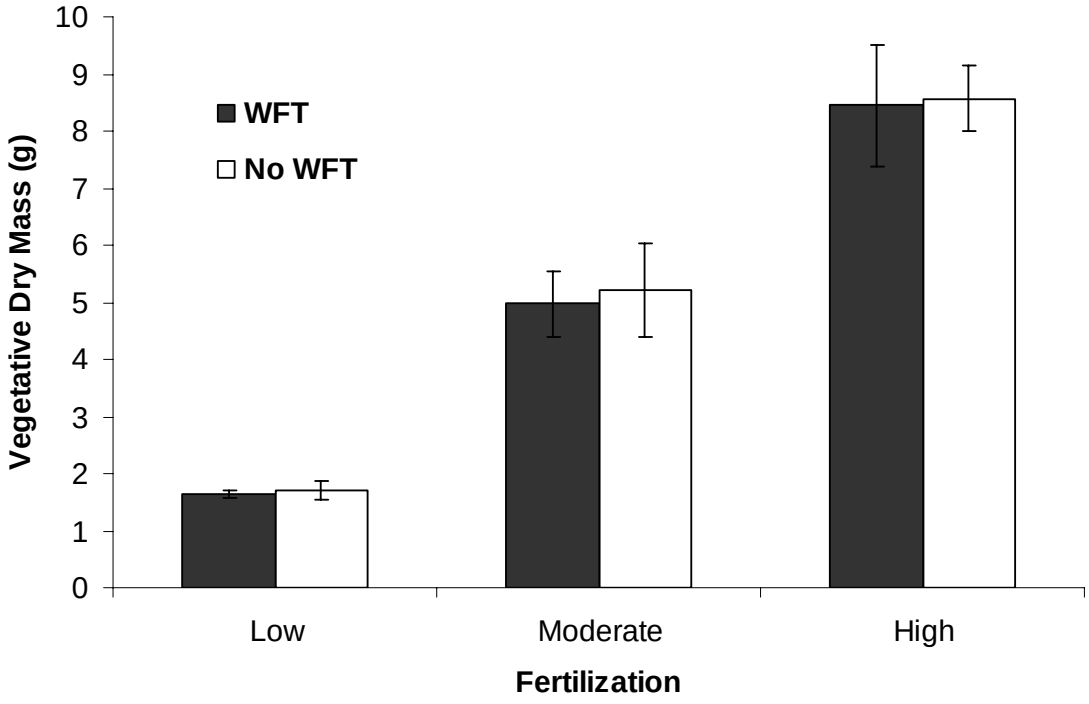

Fig. 5.2. The vegetative dry mass of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

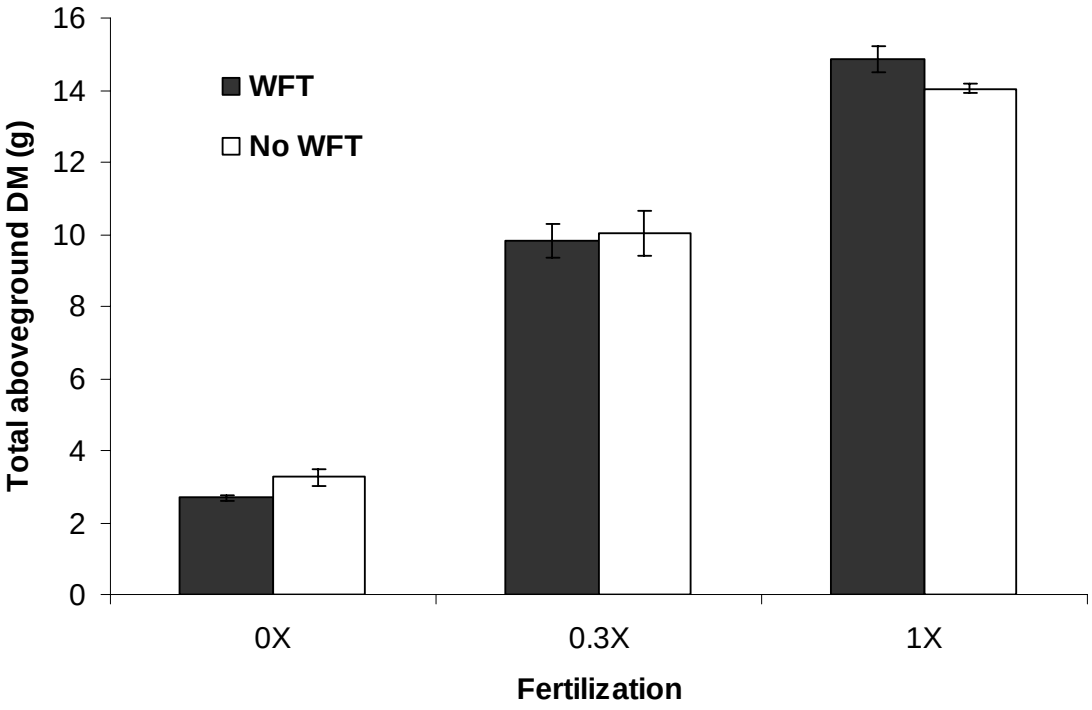

Fig. 5.3. Total aboveground dry mass (vegetative + reproductive) of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

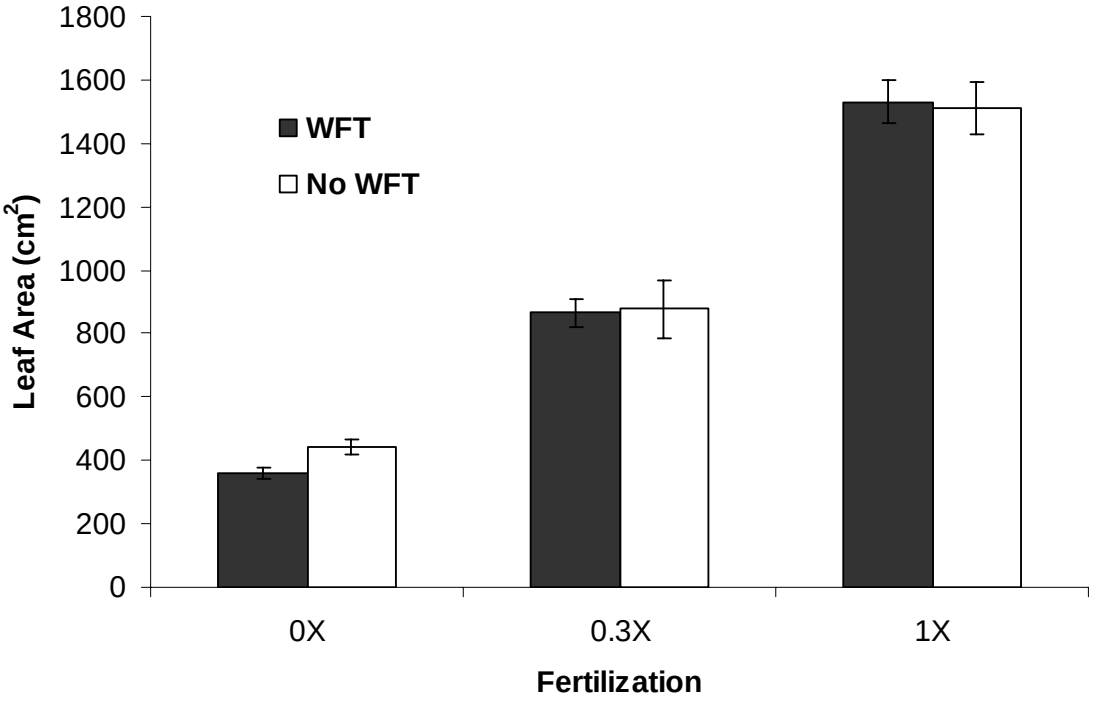

Fig. 5.4. The leaf area of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

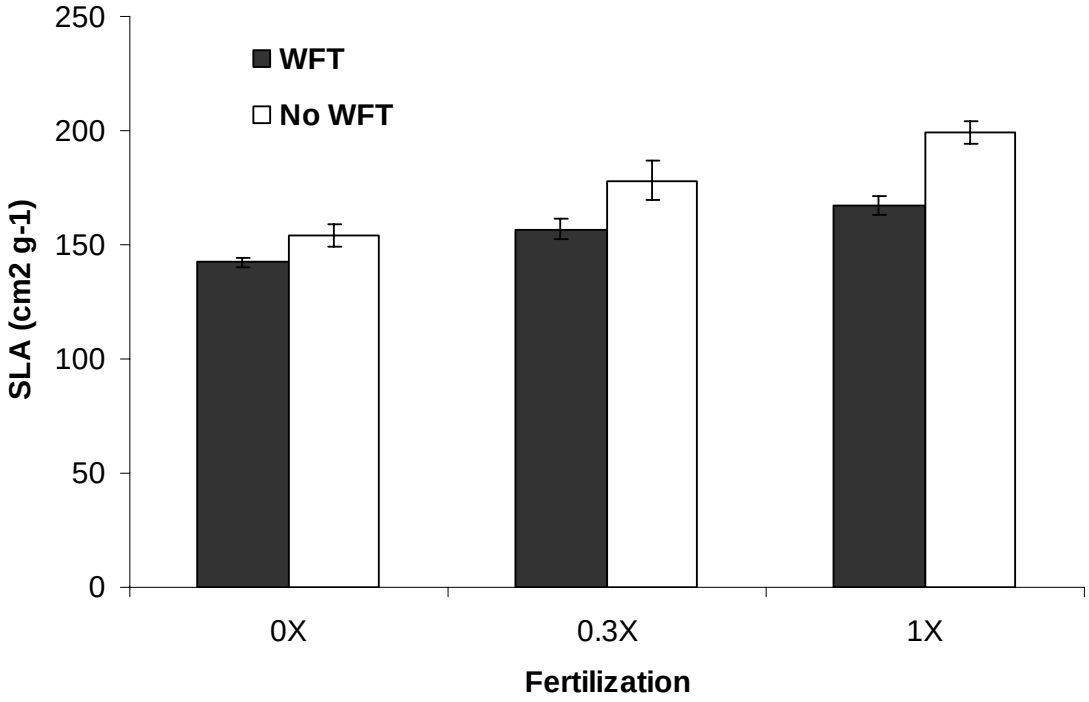

Fig. 5.5. The specific leaf area (SLA) of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of fertilization and the interaction of fertilization × WFT were highly significant ($P \leq 0.0001$), whereas WFT feeding was not significant; ± SE, n = 5.

Flower Development

Reproductive DM was reduced in 0X plants, compared to higher fertility plants ($P < 0.0001$) (Fig. 5.6). However, reproductive DM and flower DM did not differ among 0.3X and 1X plants, indicating that reducing fertilization to a moderate level affects vegetative growth more than reproductive growth in gerbera (Figs. 5.6 and 5.7). The 0.3X and 1X plants flowered and reached pollen shed at approximately the same

time (Fig. 5.8). Fertilization, WFT, and their interaction did not affect the number of days to pollen shed in 0.3X and 1X plants. However, fertilization did affect days to pollen shed in 0X plants, which were markedly nutrient deficient, and only one plant (without WFT) achieved pollen shed (data not reported).

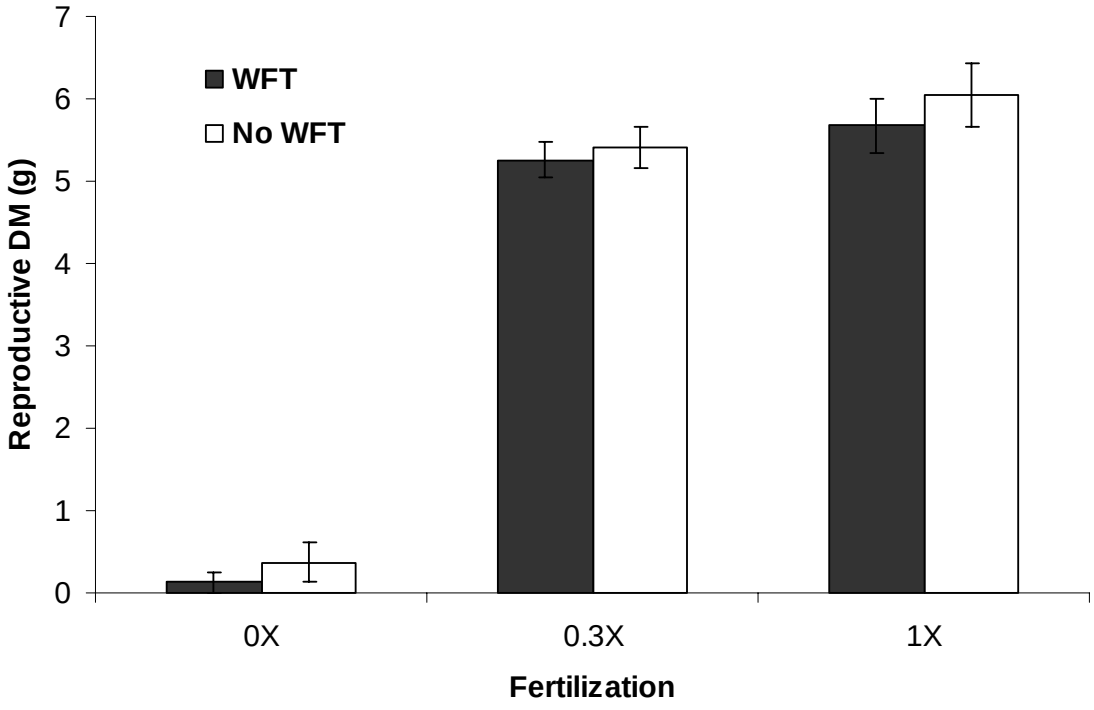

Fig. 5.6. Reproductive tissue (flowers, flower buds, peduncles) dry mass (DM) of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

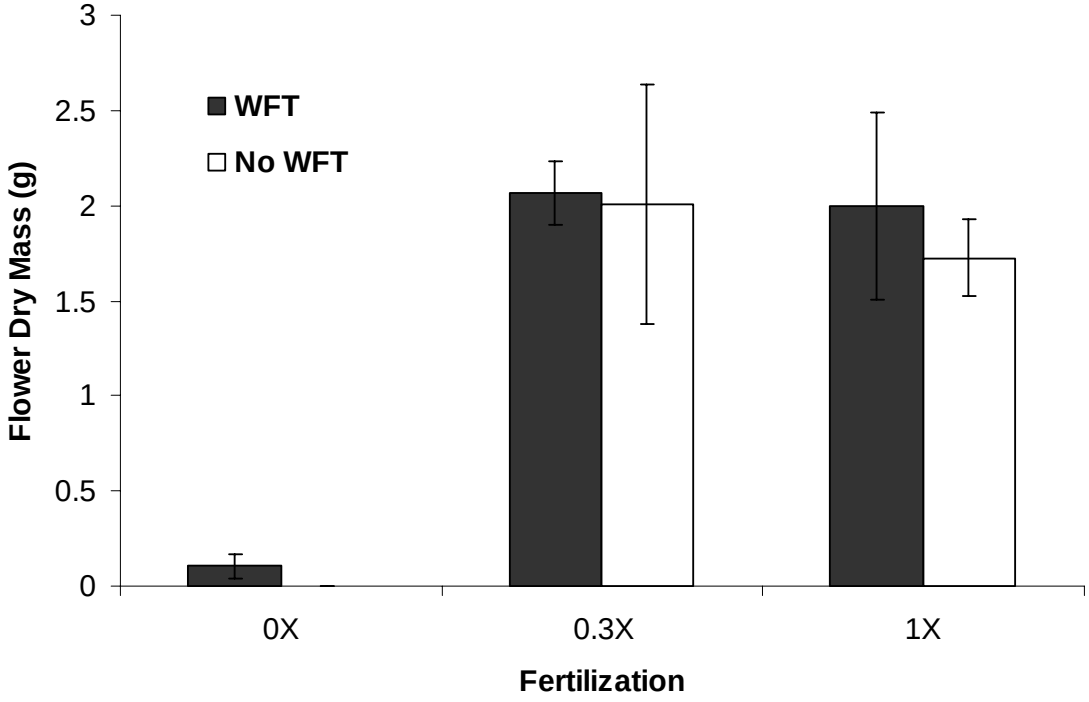

Fig. 5.7. Flower dry mass (DM) of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

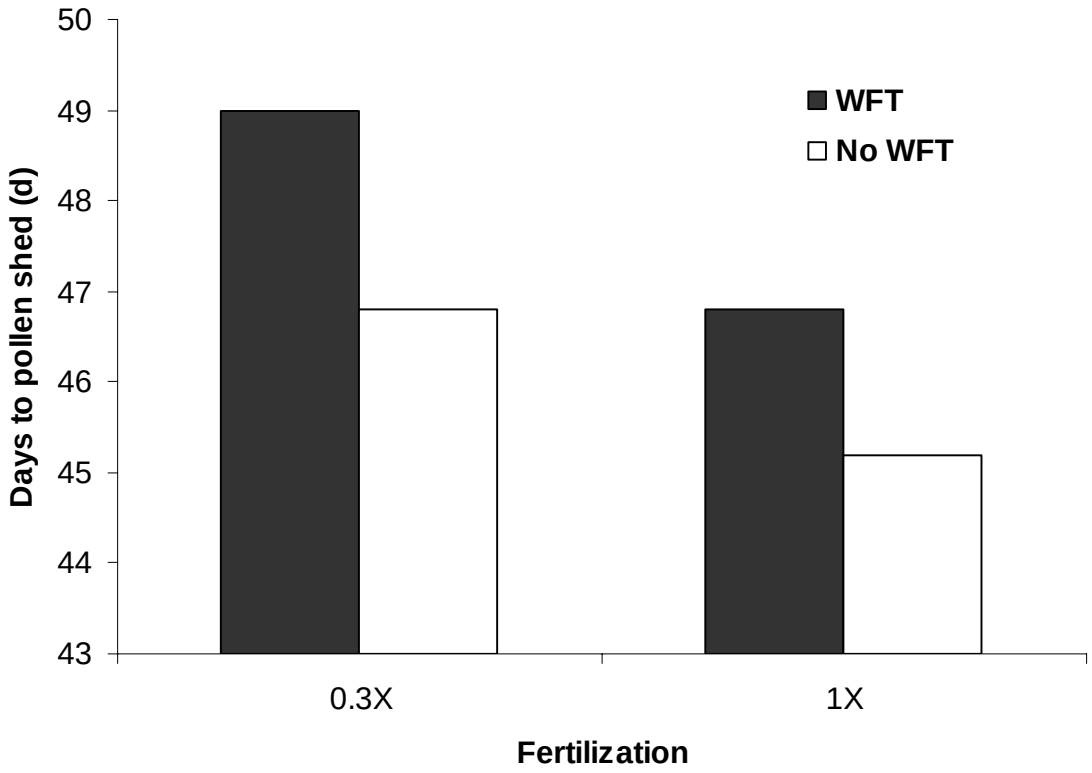

Fig. 5.8. The number of days to pollen shed for *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with two different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Low fertility plants are not included in this figure because only one plant reached pollen shed (day 49). Treatment effects of fertilization, WFT feeding, and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

It was noticed that the peduncles on 0.3X plants appeared taller compared to 1X plants, so the DM of peduncles per individual flower was determined. In response to fertilization, the DM of peduncles/flower was significantly greater on 0.3X plants compared to 1X plants ($P < 0.0001$) (Fig. 5.9). Neither WFT feeding nor the interaction affected the DM of peduncles/flower.

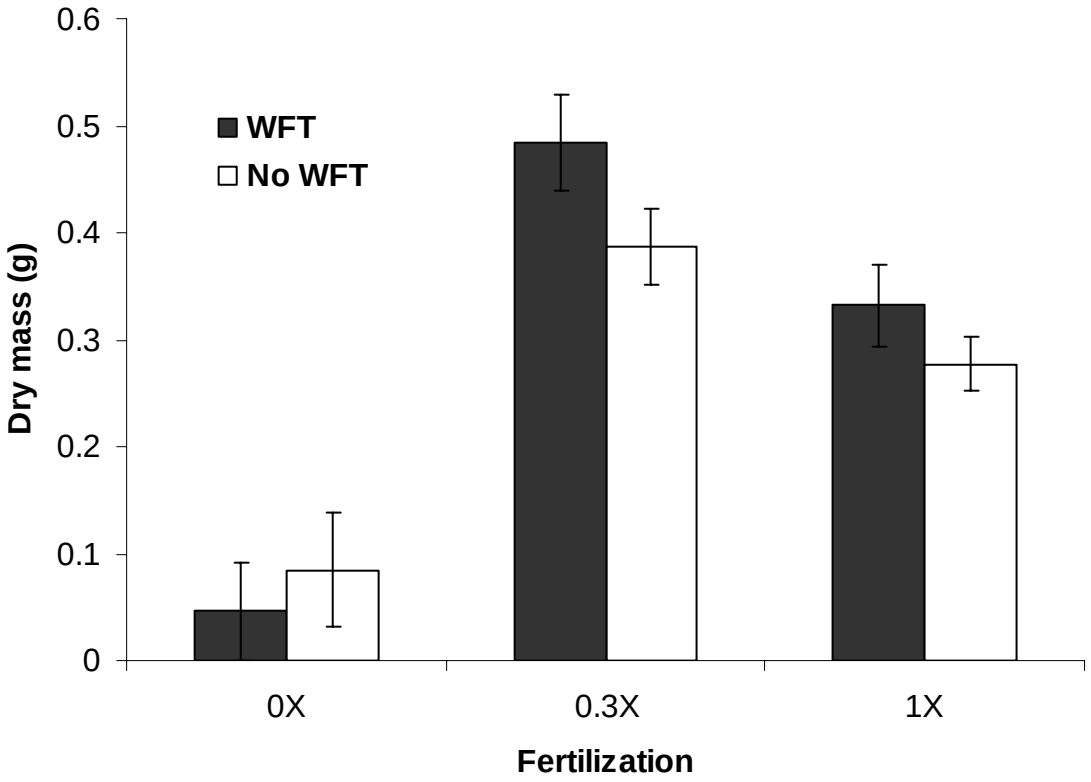

Fig. 5.9. The dry mass of peduncles per flower of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

Plant Quality

Fertilization ($P < 0.0001$), WFT feeding ($P < 0.0001$) and their interaction ($P \leq 0.0014$) had significant effects on overall plant quality (Fig. 5.10). Overall plant quality was reduced by WFT feeding on 0.3X and 1X plants. The 0X plants were noticeably stressed and had chlorotic leaf tissue and red stems. Hence, 0X plants had the lowest rating and were not marketable regardless of inoculation with WFT. The 1X plants

without WFT had the highest quality, followed by 0.3X plants without WFT. The 0.3X and 1X plants without WFT were rated as saleable (≥ 3, on scale of 1-5), whereas 0.3X and 1X plants infested with WFT were not saleable (< 3).

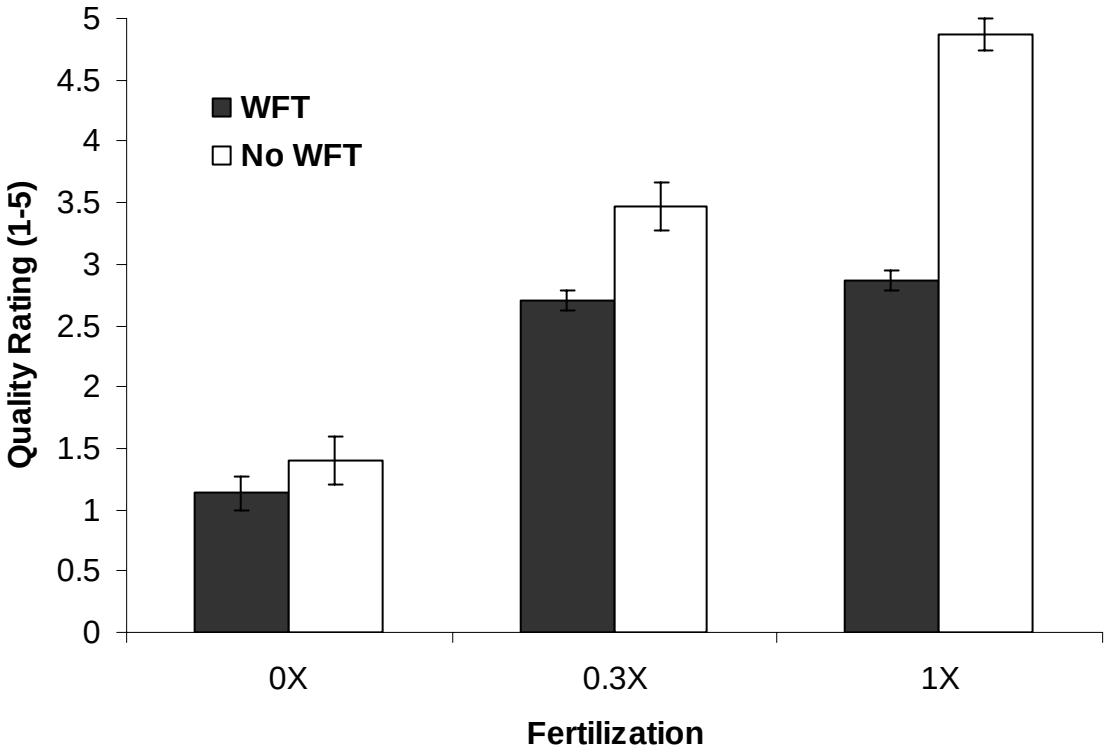

Fig. 5.10. Overall quality of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Gerberas were rated for quality on day 54, based on the following scale: 1 = very poor, unsaleable (extremely chlorotic and/or extensive WFT feeding damage); 2 = poor, unsaleable (chlorotic and/or WFT damage, few or no flowers); 3 = average, saleable (≤ 30% of media showing, lighter green and/or moderate WFT damage); 4 = good, saleable (foliage covering media, green, flowers, may have slight WFT damage); 5 = excellent, saleable (foliage covering media, dark green, no WFT damage to vegetation or flowers). Treatment effects of fertilization ($P < 0.0001$), WFT ($P < 0.0001$), and the interaction of fertilization × WFT ($P \leq 0.0014$) were highly significant; ± SE, n = 5.

Leaf Chlorophyll

Chlorophyll content in physiologically mature leaves increased due to increasing fertilizer concentrations ($P < 0.0001$) (Fig. 5.11). WFT feeding and the interaction among fertilization and WFT feeding did not affect leaf chlorophyll production.

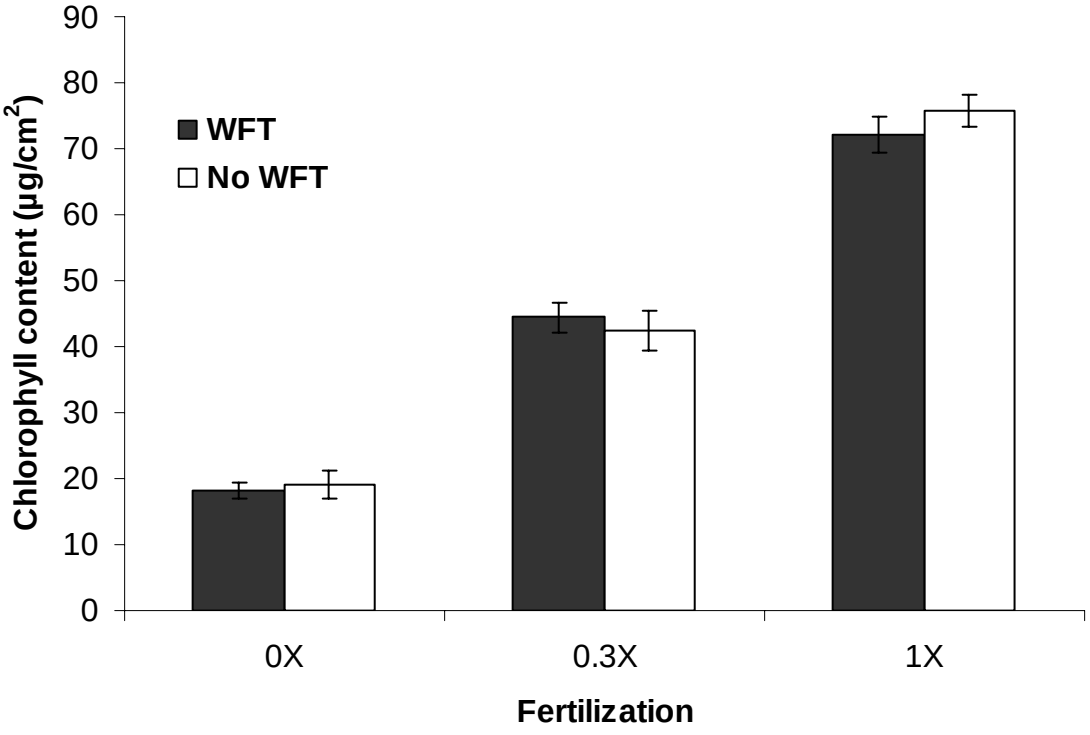

Fig. 5.11. Chlorophyll content of physiologically mature leaf tissue of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

Total Phenolic and Jasmonic Acid Concentrations

Total phenolics in leaf tissue increased in response to reduced fertilization ($P < 0.0001$) (Fig. 5.12). Total phenolics were highest in 0X plants, which had 5.3 mg g^{-1} FW, followed by 0.3X (2.7 mg g^{-1} FW) and 1X (0.8 mg g^{-1} FW) plants. WFT feeding and the interaction among WFT and fertilization were not significant.

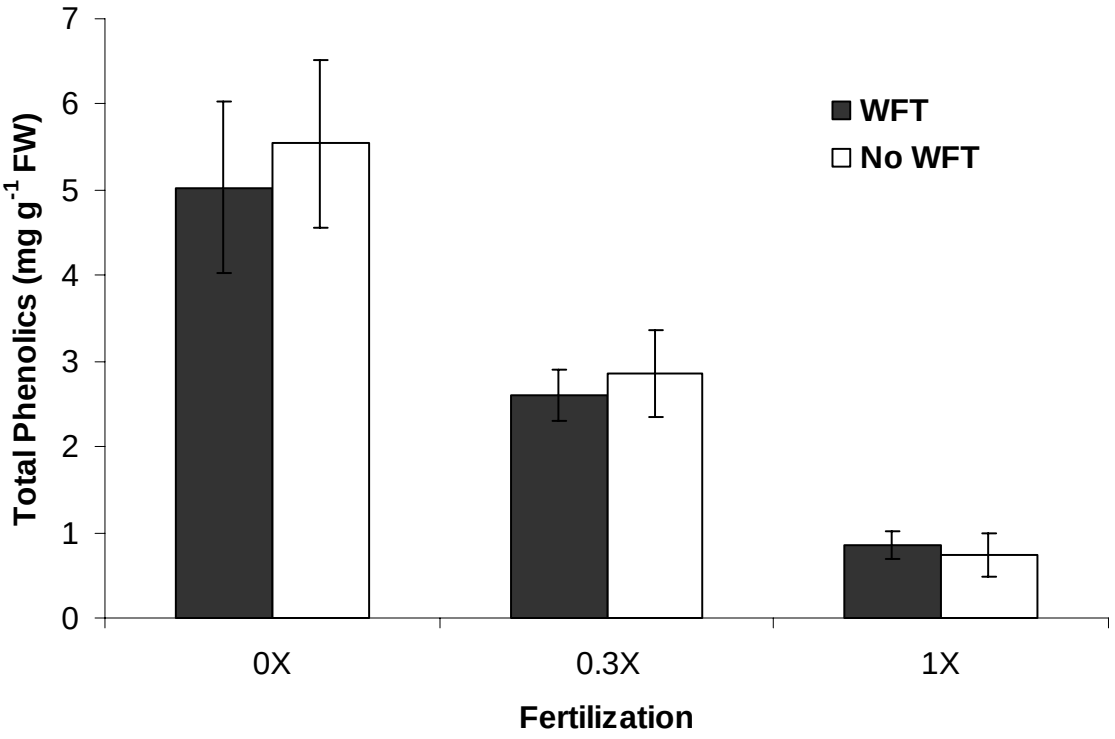

Fig. 5.12. The total phenolic concentration in physiologically mature leaves of *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) mg·L^{-1} N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

WFT feeding significantly ($P \leq 0.0481$) enhanced the accumulation of jasmonic acid in 0.3X and 1X plants (Fig. 5.13). Fertilization and the interaction among fertilization and WFT feeding did not affect JA levels. WFT feeding did not significantly affect JA levels in 0X plants. In a previous study, JA accumulation was highest 30 min after wounding in 0X plants and 1 h after wounding in 1X plants (Chapter III). While WFT enhanced JA, the effect of fertilization on WFT-induced accumulation of JA could not be determined in this study because of variability in WFT abundance and feeding among the fertility treatments.

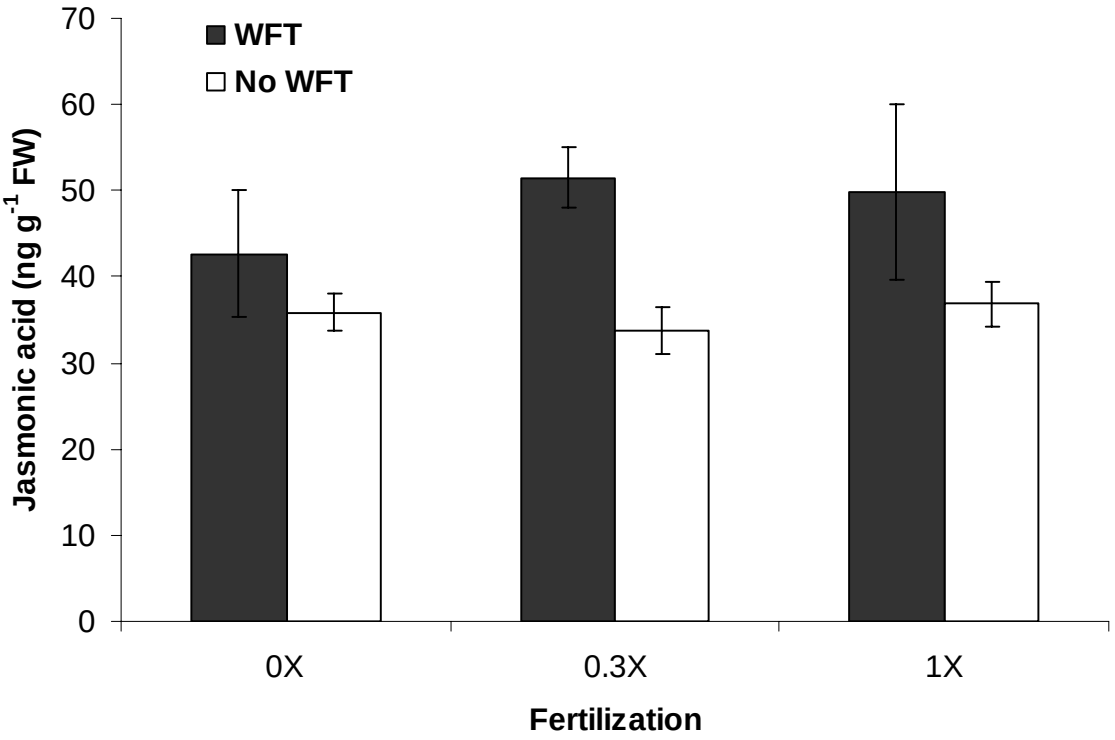

Fig. 5.13. The jasmonic acid concentration in physiologically mature leaves from *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of WFT feeding were significant ($P \leq 0.0481$), while fertilization and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

Net Photosynthesis and Stomatal Conductance

Net photosynthesis and stomatal conductance increased as fertilizer concentration increased ($P < 0.0001$) (Figs. 5.14 and 5.15). Photosynthesis ranged from 2.1 μmol CO_2 m^{-2} s^{-1} in 0X plants to12.53 μmol CO_2 m^{-2} s^{-1} in 1X plants. Neither WFT feeding, nor the interaction between fertilization and WFT feeding, had a significant effect on photosynthesis or stomatal conductance.

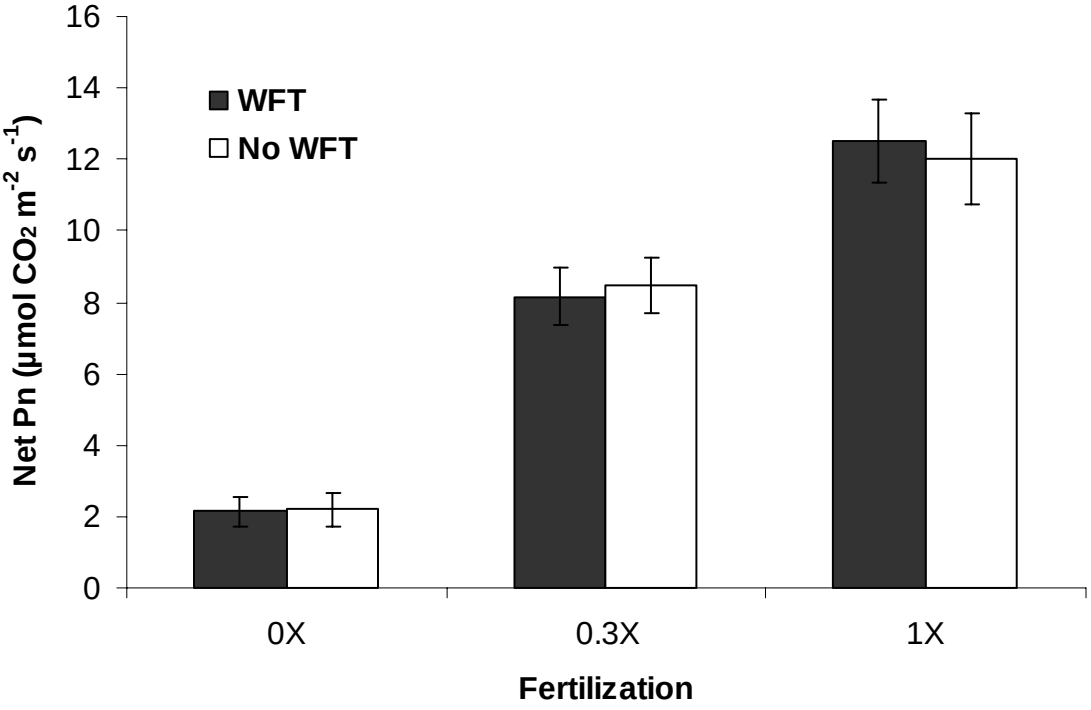

Fig. 5.14. Net photosynthesis (Pn) of physiologically mature leaves from *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) mg·L^{-1} N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

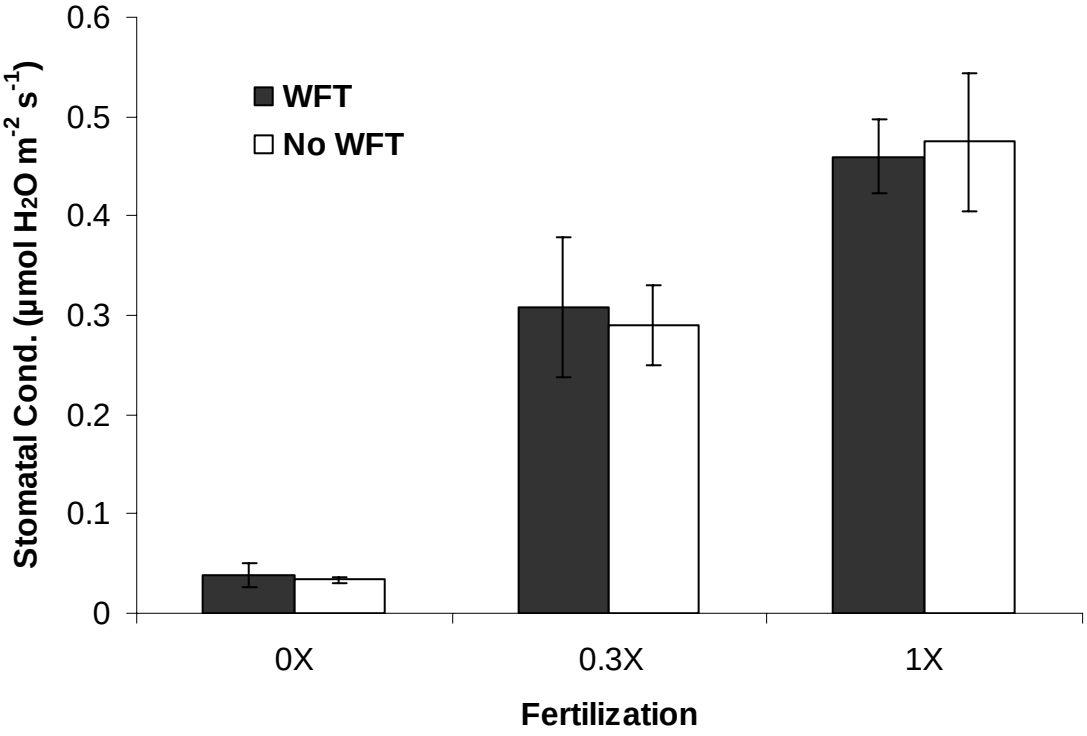

Fig. 5.15. Stomatal conductance of physiologically mature leaves from *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) mg·L^{-1} N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

Carbon/Nitrogen Ratio

The C/N ratio increased as fertilization was decreased ($P < 0.0001$) (Fig 5.16). WFT or the interaction between fertilization and WFT did not significantly affect the C/N ratio. The % C (mean = 44.2%) among gerbera plants under different fertilizer rates was not significantly different; however, the mean % N for physiologically mature gerbera leaves was 0.8% for 0X, 1.8% for 0.3X, and 3.9% for 1X plants.

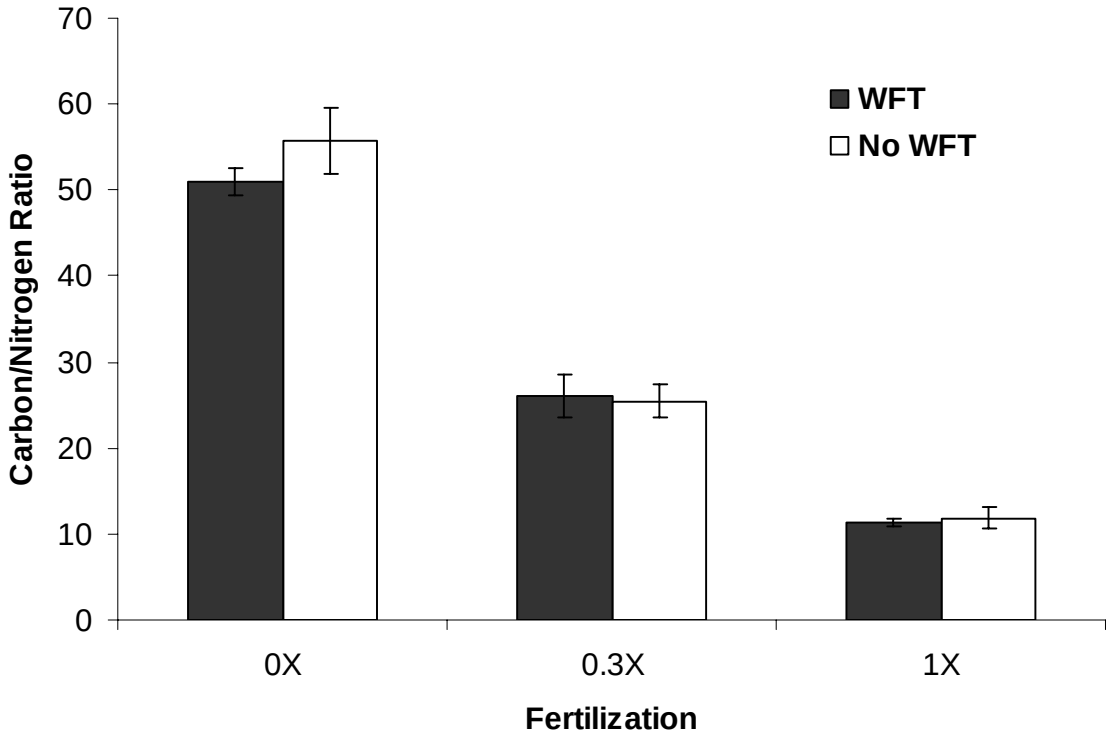

Fig. 5.16. Carbon/Nitrogen ratio of physiologically mature leaf tissue from *Gerbera jamesonii* 'Festival Salmon' inoculated with western flower thrips (WFT) or without (No WFT), treated with three different fertilizer concentrations. Plants were fertilized with 200 ml of a 15-16-17 fertilizer solution (elemental analysis: 15N-7P-14.2K) at 0 (0X), 60 (0.3X), or 200 (1X) $mg \cdot L^{-1}$ N at each watering, as needed. Treatment effects of fertilization were highly significant ($P < 0.0001$), while WFT treatment and the interaction of fertilization × WFT were nonsignificant; ± SE, n = 5.

Discussion

This is one of the first reports on the susceptibility of gerbera to WFT – detailing the influence of fertility on WFT abundance, plant growth, quality, photosynthesis, and endogenous compounds implicated in defense against phytophagous insects. A positive relationship between WFT (*Frankliniella occidentalis*) response and increased fertilization has been reported in several studies (Chau and Heinz, 2006; Davies et al., 2005; Stavinsky et al., 2002), and these results are generally attributed to the increased nutritive value of high fertility plants to WFT. Our previous research indicates that reducing nutrient availability increases plant compounds implicated in defense against insect herbivores, and this increase in chemical defenses is realized in reduced WFT feeding (Chapters III and IV). The objectives of this research were to determine the effects of fertility on WFT abundance in potted gerbera production, and the subsequent effects on plant physiology and quality.

Susceptibility to WFT was reduced in gerberas fertilized with rates that represent 0% and 30% of the recommended fertilization rate. The 0X gerbera plants, which received only initial fertilizer charge in commercial media, were noticeably stressed, which caused a shift in carbohydrates from primary metabolism to secondary metabolism, as determined by the lower plant biomass and higher phenolics compared to higher fertility plants (Herms and Mattson, 1992). There were very few WFT that survived on 0X plants, likely due to both the higher concentrations of defensive compounds (e.g. phenolics) and the low nutritional value of the 0X tissue (Mattson,

1980). However, the 0X plants were not marketable and growing plants with nutrient deficiencies is not feasible for production practices. The 0.3X gerberas, which received 30% of recommended rate, also had reduced biomass and greater phenolic concentrations compared to 1X (recommended fertility) plants—but these plants were not as stressed, and 0.3X plants without WFT were rated as marketable. Reducing fertilization by 70% (0.3X plants) did not affect flower dry mass (DM) or the rate of flowering (days to pollen shed)—and actually the flower stalks (peduncles) were taller in response to the fertilizer reduction. On the other hand, leaf area, chlorophyll content, photosynthesis, stomatal conductance, vegetative DM, and total aboveground DM were reduced in 0.3X (and 0X) plants, compared to 1X plants. The 0.3X plants without WFT were not of as high a quality as the 1X plants without WFT—due mostly to the greenness of the leaves, as also indicated by lower chlorophyll levels and reduced plant quality ratings.

In contrast to this study with gerbera, WFT populations caused a reduction in reproductive and vegetative growth in chrysanthemum, particularly at higher fertilizer levels (Davies et al., 2005). The reduction in plant biomass was due, in part, to a reduction in stomatal conductance and photosynthesis caused by WFT feeding. WFT feeding did not cause a reduction in photosynthesis or stomatal conductance with gerbera, possibly because the number of total WFT was much lower (77.7 on gerbera compared to 560.3 on chrysanthemum at recommended fertilization rates). Also in contrast to this study of gerbera, WFT infestations on chrysanthemums caused an increase in specific leaf area (thinner leaves) at higher fertility, while WFT feeding

decreased leaf area (Davies et al., 2005). In this study, specific leaf area (SLA) was higher in gerbera plants not infested with WFT, and leaf area was not affected by WFT feeding. A higher SLA indicates that fewer leaf mesophyll cells develop and that biomass is reduced per unit leaf area (Hunt and Lloyd, 1987; McDonald, 1990). SLA is frequently analyzed in comparative plant ecology, and is typically an indication of the relative growth rate (RGR), especially in fast-growing species (Shipley and Almeida-Cortez, 2003). A higher SLA usually correlates to a higher RGR, indicating that the plant is diverting photosynthates toward growth instead of leaf construction costs (Atkin et al., 2006). Thinner leaves (higher SLA) will capture more light (photosynthetically active radiation) per unit mass than a leaf with low SLA (Poorter, 2002). Thicker leaves (lower SLA) are an indication of stress, i.e. drought, hypoxia (He et al., 2007). In gerbera, it appears that the strategy is to increase leaf structure to compensate for WFT feeding.

The observed decrease in WFT abundance on lower fertility gerberas is likely due, in part, to an increase in secondary metabolites involved in defense against insect herbivores. The total phenolic concentration was increased in 0X and 0.3X plants, which supports results obtained from a previous fertilization study conducted with gerberas (Chapter III). Phenolics are the dominant allelochemicals in many plants and are known to decrease insect growth, development and survival (English-Loeb et al., 1997; Isman and Duffey, 1982; Stamp 1990; Wilkens et al., 1996). As in our study, Inbar et al. (2001) reported a negative association between plant growth and chemical defense, which supports the growth-differentiation balance hypothesis (GDBH),

described by Herms and Mattson (1992). The GDBH is premised upon a physiological trade-off between growth and differentiation processes. Secondary metabolism is physiologically constrained in dividing and enlarging cells, and requires photosynthates that could be used for growth processes. Plants have limited resources to support their physiological processes, and all requirements cannot be met simultaneously. Hence, trade-offs occur among growth, storage, reproduction and defense (Lambers and Poorter 1992). Inbar et al. (2001) reported a positive association between insect performance and plant growth. Plants subjected to nutrient stress had higher levels of total phenolics and peroxidase, which negatively affected the prolificacy of insect pests (whitefly, leafminer, and corn earworm).

Similar to our previous work, JA levels increased in response to WFT feeding in all fertility treatments. Previously, JA accumulation was proportionate to the amount of damage caused by WFT feeding (Chapter IV). The 0X fertility gerberas accumulated wound-induced JA more rapidly and this increase was sustained longer than in 1X gerberas (Chapter III). Also, 0.3X gerberas accumulated more JA relative to the amount of WFT feeding than 1X plants (Chapter IV). JA accumulates in response to physical damage to cell walls (often caused by herbivores) (Creelman and Mullet, 1997), and JA biosynthesis has been shown to be necessary for defense against both chewing and cell-content feeding (e.g. WFT) insects (Li et al., 2002). JA alters gene expression resulting in the accumulation of defense proteins such as proteinase inhibitors, which affect the digestive system of attacking herbivores (Farmer and Ryan, 1992; Reinbothe et al., 1994), or in the accumulation of low molecular weight compounds with antibiotic

properties (phytoalexins) (Blechert et al., 1995). In addition to the role of JA in direct defense against insect herbivores, JA accumulation may also function as an indirect defense. JA treated plants have been shown to increase volatile emissions that attract natural enemies, thus increasing the mortality rate of particular insect pests in gerbera (Gols et al., 1999), maize (Ozawa et al., 2004), native tobacco (Halitschke et al., 2000; Kessler and Baldwin, 2001), tomato (Thaler, 1999), and rice (Lou et al., 2005). Hence, if wound-induced JA accumulation is increased in lower fertility plants, plants may be better prepared to defend against insect pests either directly (e.g. increasing defensive compounds), or indirectly (by attracting natural enemies).

In addition to an increase in defensive compounds, reducing fertilization likely reduces the amount of nutrients available for insect herbivores. While the nutritional requirements of WFT are not well understood, there is evidence that WFT favor a high protein diet. Pollen increases the rate of growth, development, and fecundity for WFT (Hulshof et al., 2003; Trichilo & Leigh, 1988; Ugine et al., 2006a), and it has been suggested that manipulating the abundance of this resource could have widespread implications for managing thrips populations (Ugine et al., 2006b). Pollen has comparatively high concentrations of nitrogen (Slansky and Scriber, 1985), and preference for this tissue is often cited as a means of obtaining a higher protein diet (Kirk, 1997). The effects of fertilization on aromatic amino acids may also play a role in WFT activity. Mollema and Cole (1996) found consistent relations between aromatic amino acid concentrations (phenylalanine and tyrosine) of foliar tissue and the degree of damage caused by WFT. Higher fertilization rates increased the concentration of

phenylalanine in tomato flowers, and this increase was highly correlated with WFT abundance (Brodbeck et al., 2001).

In this study, 0.3X and 1X plants flowered and reached pollen shed at approximately the same time. Hence, the availability of pollen does not account for the observed differences in WFT populations. Fertilization, WFT, and their interaction did not affect the number of days to pollen shed in 0.3X and 1X plants (Fig. 5.8). However, fertilization did affect days to pollen shed in 0X plants, which were markedly nitrogen (N) deficient, and only one plant (without WFT) achieved pollen shed. Tissue N levels for high quality gerberas are generally recommended to be 2.7 – 4.1% (Dole and Wilkens, 1999). In this study, the mean % N in physiologically mature leaves was 0.8% for 0X, 1.8% for 0.3X, and 3.9% for 1X plants.

Most WFT were unable to survive on the 0X plants – three out of five 0X plants inoculated with WFT had no surviving WFT at the termination of the experiment. Similar to previous observations, dead WFT were observed on leaf surfaces of the 0X plants. Previously, WFT were unable to survive on 0X gerbera plants, and no detectable WFT feeding was observed (Chapter IV). Apparently, 0X plant tissue was toxic (due to high phenolic concentration) and/or did not provide the nutritional requirements necessary for WFT survival. In addition, there were no flowers (or pollen) available as a protein source. When other protein sources are scarce, WFT may supplement their diet by feeding on thrips larvae (Van Rijn et al., 1995). However, there is no documented evidence that WFT feed on adult WFT. The observed results are likely due to a combination of increased resistance mechanisms and the reduced nutritional value

present in leaf tissue of lower fertility plants. The 0.3X fertilized plants also had reduced WFT populations, but no mortality was observed. Likely, the prolificacy of WFT was reduced, but survival was not affected by feeding on 0.3X plant tissue. Also, pollen eventually became available on 0.3X plants which likely increased WFT fecundity.

The threshold for WFT in potted gerbera production is very low because of the unsightly damage caused by their feeding. WFT feeding reduced the quality of the gerbera plants, especially in higher fertility plants, and rendered them unsaleable according to our rating system. When WFT feed on expanding tissues, the tissue becomes distorted because the affected cells are unable to expand further. The leaves of WFT-infested plants had characteristic 'silver' scars, because the cells were full of air (De Jager et al., 1995). Once the plants started flowering, WFT moved into the flower buds and resulting flowers were severely distorted.

Lowering fertilization by as much as 70% of the recommended rate may be more suited for cut flower production. In potted gerbera production, it is desirable for the foliage to cover the media, for the leaves to be dark green, and for the flowers to be just above canopy level. Obviously in cut flower production, the foliage is not as much of a concern, and long flower stalks (peduncles) are desirable. Hence, in addition to increased host plant resistance, the longer peduncles in the 0.3X gerberas would be an added benefit. Even though WFT populations were reduced by lowering fertilization, they were still not at an acceptable level in the 0.3X plants. Ideally, reducing fertilization would just be one strategy that is incorporated into a comprehensive IPM system. Gerbera cut flower IPM programs are already in use in some nurseries, in which

a variety of insect predators are used for biological control in conjunction with biorational insecticides (Newman, 2004). Reducing fertilization may be another tool that can be used to keep pest populations low and reduce pesticide applications, while maintaining high quality.

Summary

This research focused on the influence of fertilization on plant growth and development, overall quality, western flower thrips (WFT) abundance, and host plant resistance characteristics of gerbera (*Gerbera jamesonii* 'Festival Salmon'). We tested three fertility levels that consisted of 0%, 30%, or 100% (200 mg L^{-1} N) the recommended fertilization rate for gerbera. Each pot, with one established seedling, was enclosed in a large acetate cylindrical cage and half were inoculated with five adult female WFT [*Frankliniella occidentalis* (Pergande)]. Susceptibility to WFT in gerbera was reduced using fertilization rates that represent 0% (0X) and 30% (0.3X) of the recommended fertilization rate. WFT abundance was severely reduced on the lowest fertility plants—which received only the initial fertilizer charge present in the media. Higher rates of fertilization increased plant quality, chlorophyll content, vegetative DM, total aboveground DM, photosynthesis, stomatal conductance, and leaf area. Flower production and the number of days to pollen shed were similar for gerberas receiving 0.3X and 1X (100%) of the recommended fertilization, but much lower for plants receiving 0X fertilization. WFTs reduced the overall plant quality (marketability) of

gerberas receiving 0.3X and 1X fertilization, but had no apparent affect on vegetative and reproductive growth, photosynthesis, or stomatal conductance. WFT-free plants had increased specific leaf area (i.e., thinner leaves), indicating a higher relative growth rate. Phenolics, which are constitutive secondary metabolites that have been shown to negatively affect insect feeding, increased as fertilization was reduced. WFT feeding did not affect phenolic content, but did induce the accumulation of the phytohormone, jasmonic acid—which is known to regulate inducible defenses against insect herbivory. Reducing fertilization by 70% in gerbera production, which also increased flower peduncle length, may increase resistance to WFT, while maintaining marketable plant quality.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary

Plants use constitutive and induced defenses to aid in protection against phytophagous insects. Nutrient availability has a substantial effect on both of these resistance mechanisms. When nutrient limitations reduce growth, allocation to secondary metabolism is favored, and total phenolic concentrations within the plant are typically increased. Phenolics are the dominant allelochemicals in many plants and are known to decrease insect growth, development and survival (English-Loeb et al., 1997; Isman and Duffey, 1982; Stamp 1990; Wilkens et al., 1996). Jasmonates are the best-characterized class of signals mediating the elicitation of defense responses to wounding and herbivory, and they have been shown to be necessary for the induction of a wide array of defensive compounds. However, there is limited information on the effects of nutrient availability on jasmonic acid (JA) accumulation –just two studies that reported contrasting effects of N deficiency on JA.

A goal of this research was to determine the effects of fertilization on constitutive defense (i.e. phenolics) and induced defense (i.e. JA) mechanisms present in gerbera. The floriculture crop *Gerbera jamesonii* is an economically important crop with substantial fertilization recommendations. Gerbera is also highly susceptible to a variety

of insect pests, of which western flower thrips [(WFT) *Frankliniella occidentalis* (Pergande)] are the most problematic. Hence, pesticide use on gerbera is prevalent. In order to ascertain the practicality of altering fertilization to increase host plant resistance, the effects of fertilization on WFT abundance and feeding, and the subsequent effects on plant quality, physiology, growth and development was determined.

The first study (Chapter III) was conducted to determine effects of fertilization on constitutive total phenolic and jasmonic acid accumulation in gerbera. This was the first study to report JA accumulation in gerbera. In response to mechanical damage, JA accumulation increased more rapidly and the accumulation was sustained longer in low fertility [(0X) only received fertilizer charge present in professional growing mix] plants compared to recommended fertility (1X) plants. In 0X plants, JA levels peaked 0.5 h after wounding, and JA accumulation was significantly higher 0.5 h and 3 h after wounding, compared to 1X plants. JA accumulation peaked 1 h after wounding in 1X plants, but this level was not significantly higher than 0X plants. Physiologically mature leaves of 0X plants had approximately a 10-fold higher concentration of total phenolics, when compared to 1X plants. In 0X and 1X plants, young leaves had greater concentrations of phenolics compared to physiologically mature leaves. There were no differences in total phenolics due to wounding.

The second study (Chapter IV) evaluated the effects of fertilization on western flower thrips [(WFT) *Frankliniella occidentalis* (Pergande)] feeding, and the subsequent effects on JA accumulation. This was one of the first studies to determine that WFT feeding induces the accumulation of jasmonic acid (JA). JA levels increased in response

to WFT feeding on 0.3X (received 30% of recommended fertilization) and 1X treatments. WFT feeding damage was substantially reduced on 0X and 0.3X plants. In fact, WFT were unable to survive on 0X gerbera plants, and no detectable WFT feeding was observed. JA accumulation was proportionate to the amount of damage caused by WFT feeding. The 0.3X plants accumulated substantially more JA relative to the amount of WFT feeding damage (47.7 ng g^{-1} FW for 1 percent increase in PFD), compared to 1X plants (7.7 ng g^{-1} FW for 1 percent increase in PFD). In addition to WFT-induced JA accumulation, constitutive levels of JA were also affected by fertilization. JA levels were significantly higher in 0X plants, when compared to 1X plants, though constitutive JA levels in 0.3X plants were not significantly different compared to 0X or 1X plants.

The third study (Chapter V) was conducted to determine the effects of fertilization on WFT abundance, and to characterize the effects of fertilization and WFT feeding on various plant growth and physiological characteristics. In addition, JA accumulation was measured to determine whether WFT feeding induces JA biosynthesis. The effects of fertilization and WFT feeding on gerbera plant quality was also determined to assess the viability of altering fertilization in order to increase host plant resistance in gerbera. Susceptibility to WFT in gerbera was reduced using fertilization rates that represent 0% (0X) and 30% (0.3X) of the recommended fertilization rate. There were very few WFT that survived on the 0X plants; however, the 0X plants were not marketable and growing plants with nutrient deficiencies is not feasible for production practices. Similar to 0X plants, the 0.3X fertilized gerberas had

reduced biomass and greater phenolic concentrations compared to 1X plants, but these plants were not as stressed, and 0.3X plants without WFT were rated as marketable. Reducing fertilization by 70% (0.3X plants) did not affect flower dry mass (DM) or the rate of flowering (days to pollen shed), and actually the flower stalks (peduncles) were higher in response to the fertilizer reduction. Higher rates of fertilization increased plant quality, chlorophyll content, vegetative DM, total aboveground DM, photosynthesis, stomatal conductance, and leaf area. WFTs reduced the overall plant quality (marketability) of gerberas receiving 0.3X and 1X fertilization, but had no apparent affect on vegetative and reproductive growth, photosynthesis, or stomatal conductance. WFT-free plants had increased specific leaf area (i.e., thinner leaves), indicating a higher relative growth rate, i.e. non-stressed; stress leaves are frequently thicker. WFT feeding did not affect phenolic content, but did induce the accumulation of the phytohormone, jasmonic acid.

Conclusions

The present research demonstrated the effects of fertilization on the host plant resistance of *Gerbera jamesonii* to western flower thrips (WFT). Chemical defenses present in gerbera were enhanced when fertilization was reduced to 0% or 30% of recommended concentrations. Reducing fertilization increased total phenolics (constitutive defense) and wound- and WFT-induced JA accumulation in gerbera. This

increase in chemical defenses reduced the prolificacy of WFT; as determined by less WFT feeding and abundance in lower fertility plants.

Based on the results of this study and previous research on the effects of fertilization on endogenous chemical defenses (Inbar et al., 2001; Schmelz et al., 2003b; Stout et al., 1998), the demand for plant defense signaled by insect feeding may override physiological constraints imposed on growth. This indicates that the strategy for some plant species under nutrient stress is to increase constitutive defenses, while maintaining, or possibly increasing inducible defenses instead of growth.

WFT were often unable to survive on nutrient-deficient 0X fertility plants (received only initial fertilizer charge present in growing mix); however, these plants were noticeably stressed and not marketable. However, 0.3X fertility gerberas (received 30% of recommended fertilization) had similar flower production compared to gerberas receiving recommended fertilization rates, and were rated as marketable. Hence, reducing fertilization may be a viable tool in commercial gerbera production that enhances host plant resistance to WFT, while maintaining marketable quality. If fertilization is reduced to a level that increases host plant resistance and maintains plant quality—fertilizer run-off, pesticide usage, and associated chemical phytotoxicity could also be reduced in greenhouse production systems.

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